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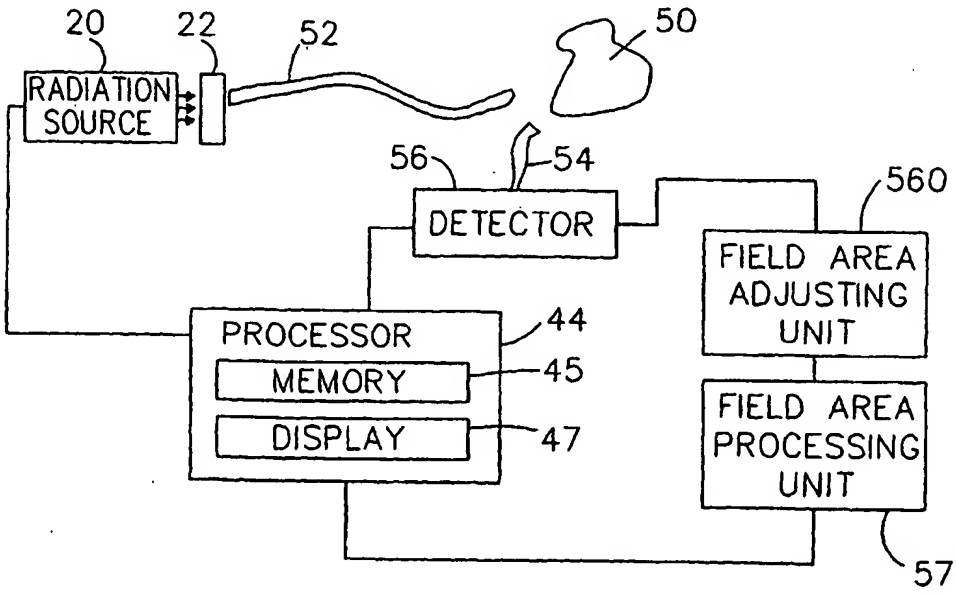
(54) Title: MULTI-MODAL OPTICAL TISSUE DIAGNOSTIC SYSTEM



(57) Abstract: An apparatus and method according to the invention utilizes a radiation source (20) and processor (44) to combine more than one optical modality (spectroscopic method), including but not limited to fluorescence, absorption, reflectance, polarization anisotropy, and phase modulation, to decouple morphological and biochemical changes associated with tissue changes due to disease, and thus to provide an accurate diagnosis for tissue condition.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MULTI-MODAL OPTICAL TISSUE DIAGNOSTIC SYSTEM

FIELD OF THE INVENTION

The invention relates to apparatus and methods for determining tissue characteristics of biological tissue.

SUMMARY OF THE INVENTION

The invention focuses on providing methods and apparatus that provide accurate measurements of changes in characteristics of tissues. The methods and apparatus according to the invention combine more than one optical modality (spectroscopic method), including but not limited to fluorescence, absorption, reflectance, polarization anisotropy, and phase modulation to decouple morphological and biochemical changes associated with tissue changes, and thus to provide an accurate diagnosis of the tissue's condition. The measurements taken according to the various spectroscopic methods can be equally weighted for diagnostic purposes, or can be weighted in various manners to produce the best diagnostic results. For example, the results may be weighted based on characteristics particular to the tissue subject, such as, for example, patient ages, hormonal metabolism, mucosal viscosity, circulatory and nervous system differences.

The invention encompasses apparatus and methods for determining characteristics of target tissues, wherein excitation electromagnetic radiation is used to illuminate a target tissue and electromagnetic radiation returned from the target tissue is analyzed to determine the characteristics of the target tissue. Some apparatus and methods embodying the invention can be used to perform a diagnosis at or slightly below the surface of biological tissue. For instance, methods and apparatus embodying the invention could be used to diagnose the condition of skin, the lining of natural body lumens such as the gastrointestinal tract, or the surfaces of organs or blood vessels. Other apparatus and methods embodying the invention can be used to perform a diagnosis deep within tissues of, for example, a human or

animal, where the excitation radiation has to pass through several centimeters of tissue before it interacts with the target tissue, such as in diagnosis of tumors and lesions deep in a breast of a human or animal.

According to one embodiment of the invention, an apparatus and method are provided which utilize fluorescence in combination with reflectance in order to decouple the biochemical changes from the morphological changes. The fluorescence and reflectance information may be separately analyzed and compared, or alternatively, can be calibrated to take into account the attenuation due to absorption and scattering. Other combinations of spectroscopic methods besides fluorescence and reflectance may also be appropriate.

Measurements using the various spectroscopic methods may be taken simultaneously, or may be taken one after the other.

The above described techniques can be used to determine the characteristics of a single portion of a target tissue, or to determine characteristics of multiple portions of the target tissue. If multiple portions are to be analyzed, the target tissue may be analyzed as a whole by simultaneously taking measurements at a plurality of interrogation points on the tissue surface, or by taking measurements at only a portion of the plurality of interrogation points covering substantially the entire tissue surface at timing intervals until measurements have been taken at all of the plurality of interrogation points.

Further, the target tissue can be divided into a plurality of field areas to create a field pattern. Measurements may then be taken at one or more points within each of the field areas. The field areas may be then separately analyzed and compared in order to diagnose a condition of the target tissue. The target tissue can then be redivided into a different set of field areas and the field areas analyzed and compared in order to diagnose the condition of the tissue. The field areas may be all identically sized and/or shaped, or may have varied sizes and/or shapes. Further, the target tissue may be redivided into field areas of the same size and shape as the

original field areas, which then are merely repositioned, or it may be redivided into field areas of a different size and/or shape, or of varied sizes and/or shapes.

As discussed above, techniques embodying the invention can be used to determine the conditions of multiple portions of a target tissue, and the determined conditions can be used to create a map of the target tissue. Such a map could then be either displayed on a display screen, or presented in hard copy format.

Further, the techniques can be used to feed information into a pattern recognition algorithm, or neural network.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described with reference to the following drawing figures, wherein like elements are referred to with like reference numerals, and wherein:

Figure 1 is a schematic diagram showing an apparatus embodying the invention capable of performing a phase shift measurement;

Figure 2 is a schematic diagram of an endoscope embodying the invention;

Figures 3A and 3B show another embodiment of the invention;

Figures 4A, 4B and 4C show the end portions of various embodiments of the invention;

Figure 5 is a cross-sectional view of another embodiment of the invention;

Figures 6A and 6B are alternative cross-sectional views of the apparatus of Figure 5 taken along section line 10-10;

Figures 7A-7D, 8 and 9 show various arrangements of optical fibers;

Figure 10 shows another embodiment of the invention;

Figure 11A is a schematic diagram showing another embodiment of the invention;

Figures 11B - 11D show how target tissue can be divided into a plurality of field areas;

Figure 12 shows the steps of a method embodying the invention;

Figures 13 and 14 are ROC plots comparing the results obtained according to the invention to results obtained using pap smear testing and/or the line of chance;

Figures 15A and 15B are false color fiber optic maps of the results obtained for two patients according to the invention; and

Figures 16A and 16B are images obtained using a multi-modal hyperspectral imaging (MHI) system to implement the invention.

DETAILED DESCRIPTION OF EMBODIMENTS

The techniques according to the invention are designed to discriminate normal tissue from various dysplastic tissue stages based on spectroscopic data alone. Additional factors, such as, for example, patient age, menopausal status, menstrual state, and/or previous history of disease can be added to the spectroscopic inputs in achieving better discrimination.

The multimodal approach according to the invention may be carried out in an imaging mode. The multiple spectroscopic methods are used in interrogating tissue at several interrogation points at high spatial resolution concurrently. The reasoning behind this approach is the variability in spectroscopic signature of known normal tissue between patients and the fact that in 99% of the patients the entire organ is not diseased. The best way to do this without an *a prior* knowledge of what is normal is to measure both the normal and abnormal tissue, that is, the entire organ.

The invention will now be further discussed with reference to the drawings.

Figure 1 is a schematic diagram of an apparatus embodying the invention. The apparatus includes a source 20, which produces electromagnetic radiation that is conducted to a target tissue 50. The electromagnetic radiation could be conducted to the tissue by one or more emission optical fibers 52. The apparatus may also

include a filter 22 for controlling the electromagnetic radiation emitted from the radiation source 20. The source 20 could comprise, for example, a laser, a light emitting diode, a fluorescent tube, an incandescent bulb, or any other type of device that is capable of emitting electromagnetic radiation, as is well known to those skilled in the art.

Electromagnetic radiation returned from target tissue 50, is sensed by a detector 56. As discussed below, the detector may employ any of the known methods for determining tissue characteristics, including but not limited to fluorescence, absorption, reflectance, anisotropy, phase change, and any other know spectroscopic methods including those methods discussed in the Background of the Invention section of this disclosure. The detector employs two or more spectroscopic methods which provides for a better or more accurate measure of target tissue characteristics than one measurement alone, and thus a more complete diagnosis of the tissue's condition.

The returned electromagnetic radiation comprises both fluorescent emissions from fluorophores in the target tissue that have been excited by the excitation radiation and the excitation electromagnetic radiation that is scattered or reflected from the target tissue. In an embodiment of the invention, as later discussed, the detector 56 makes intensity based measurements on both forms of said electromagnetic radiation. These measurements are combined to decouple the morphological changes from the biochemical changes. The detector may comprise, for example, a photomultiplier tube, a photosensitive diode, a charge coupled device, or any other type of electromagnetic radiation sensor, as is also well known to those skilled in the art.

If the detector is a small charge coupled device, it could be located at a distal end of an endoscope or catheter instrument. In this instance, the charge coupled device would already be located adjacent the target tissue such that the detector

could directly sense the return radiation. The charge coupled device would then need some means for communicating its information to a processor 44.

If the detector is not a charge coupled device located at a distal end of an instrument, the returned electromagnetic radiation may be conducted to the detector 56 through one or more return optical fibers 54. The return optical fibers 54 and the excitation optical fibers 52 may be co-located within the same instrument, or they may be located in separate instruments. Alternately, the same optical fibers within an instrument may be used to perform both excitation and return functions.

The processor device 44 may include a memory 45 and a display 47. In fact, the processor device may comprise a typical personal computer.

In embodiments of the invention, the detector 56 may detect the fluorescent emissions from fluorophores in the target tissue simultaneously with the excitation electromagnetic radiation that is scattered or reflected from the target tissue to provide a complete analysis of the subject tissue. Alternatively, the device may be configured to first detect the fluorescent emissions from fluorophores in the target tissue, and then subsequently, the excitation electromagnetic radiation that is scattered or reflected from the target tissue.

In some embodiments of the invention, where the scattered/reflected electromagnetic radiation is detected separately from the fluorescent emissions, the time period between detections, hereinafter referred to as the "critical timing window," should be minimized to avoid motion artifacts and/or significant tissue changes that will denigrate the overall results. The time period between detections is preferably less than approximately 0.25 seconds; however, the smaller the time period, the more accurate the results will be.

Figure 2 shows an endoscope that could be used to practice the measuring techniques according to the invention. The endoscope 60 includes a transmit optical fiber bundle 52, which can convey excitation electromagnetic radiation from

a radiation source 20 to a target tissue. The endoscope 60 also includes a return optical fiber bundle 54 for communicating fluorescent emissions and/or reflected/scattered electromagnetic radiation from a target tissue to a detector 56. In alternative embodiments, the transmit and return optical fibers may be co-located, may be the same fibers, or may be a double set of fibers, as discussed below.

It may be helpful to make simultaneous detections at a plurality of interrogation points rather than at just one point or a minimum number of points. This allows evaluation of the field effect changes over an area of the tissue or substantially the entire tissue, as will be more fully discussed below. Taking measurements at just one interrogation point or a minimum number of interrogation points may be misleading as it may not provide a sufficient sampling of tissue area to accurately reflect the tissue's condition.

For example, the detector could be configured to make detections at a large number of interrogation points distributed over substantially the entire surface area of the subject tissue. That is, in one embodiment, the return optical fibers 54 could include a large number of optical fibers distributed to allow detections to be made at a corresponding large number of interrogation points on the tissue, covering substantially the entire surface of the subject tissue. Each of the optical fibers could transmit excitation electromagnetic radiation to the subject tissue and then return the return electromagnetic radiation to the detector 56. The tissue could be analyzed as a whole, or divided into a plurality of field areas.

Alternatively, a transmitting optical fiber and a return optical fiber could be located at each of the interrogation points (see, for example, Figure 7B). Further, each interrogation point could include a double set of optical fibers, a transmitting optical fiber and a return optical fiber for detecting fluorescence, and a transmitting optical fiber and a return optical fiber for detecting scattering or reflectance (see, for example, Figure 7C). In such a case, the optical fibers could be arranged to focus on the same point on the subject tissue (see, for example, Figure 7D).

Additionally, the apparatus may include a rotatable core 114, as discussed with respect to the embodiment of Figure 5, or alternatively, the tissue may be mounted on a rotatable table (not shown), so that the detector 56 would make at least one detection at just a portion of the multiple interrogation points. Then, either the rotatable head or the rotatable table could be rotated and the detector would make at least one detection at the next set of interrogation points. The process would continue to complete, for example, six rotations in order to cover substantially the entire surface of the subject tissue.

The endoscope 60 may also include a handle 62 for positioning the endoscope, or for operating a device 64 on a distal end of the endoscope 60 intended to remove tissue samples from a patient. The endoscope may also include a device 66 for introducing a dose of medication to a target tissue. Also, the source of electromagnetic radiation 20 may be configured to emit a burst of therapeutic radiation that could be delivered to a target tissue by the endoscope.

Figures 3A and 3B show the structure of an endoscope or catheter which may embody the invention. In this embodiment, the apparatus includes a long body portion 70 which is intended to be inserted into a body of a human or animal. The body portion 70 must have a diameter sufficiently small to be inserted into blood vessels or other natural lumens of the human or animal.

The device includes a proximal end 80, which holds proximal ends of optical fibers 72a - 72c. The optical fibers extend down the length of the device and terminate at a distal holding portion 74. The distal holding portion 74 holds the optical fibers in a predetermined orientation. The optical fibers are held such that they can illuminate selected portions of the distal end 76 of the device. This orientation also allows the distal end of the optical fibers to receive radiation from selected areas outside the distal end 76 of the device.

As seen in Figure 3B, the optical fibers are arranged such that there is a single central optical fiber 72a surrounded by a first ring of optical fibers 72b, which is in

turn surrounded by a second ring of optical fibers 72c. Of course, other orientations of the optical fibers are possible.

By applying excitation electromagnetic radiation to selected ones of the optical fibers, and monitoring the returned electromagnetic radiation through selected ones of the optical fibers, it is possible to determine characteristics of target tissues at selected locations outside the distal end of the device. For instance, if the central optical fiber 72a emits electromagnetic radiation 90 toward a target tissue, and returned electromagnetic radiation is sensed through the same optical fiber, the returned electromagnetic radiation can be analyzed using any of the above methods to determine characteristics of a target tissue located adjacent the center of the distal end of the device. The same process can be used to determine the condition of a target tissue at different locations around the distal end of the device.

Figures 4A-4C show various different distal ends of the device.

In Figure 4A, the distal ends of the optical fibers are held by a holding portion 98 that aims the distal ends of the optical fibers 97 in a particular direction. A flexible wire or bar 96 is attached to the holding portion 98 and extends to the proximal end of the device. By rotating the flexible wire or bar 96, the holding portion 98 can also be rotated. This allows the distal ends of the optical fibers to be aimed at different portions of the distal end of the device.

Figure 4B shows another embodiment of the invention that includes one or more inflatable balloon portions 92a, 92b. An optical fiber 72 is located in the center of the device by a holding portion 94. Each of the inflatable balloons 92a, 92b is also held by the holding portion 94. By selectively inflating or deflating the different balloon portions, the optical fiber 72 may be aimed to illuminate different portions of the distal end of the device or to receive return radiation from selected locations adjacent the distal end of the device.

Figure 4C shows an embodiment of the device similar to the embodiment shown in Figures 3A and 3B. This figure shows how electromagnetic radiation

passing down through the optical fibers 72a-72c can be used to selectively illuminate material or tissue adjacent selected portions of the distal end of the device. In Figure 4C, only the upper optical fibers are emitting electromagnetic radiation outside the device. This electromagnetic radiation is being used to destroy or atomize plaque which has formed on an inner wall of a blood vessel. By applying electromagnetic radiation to selected ones of the optical fibers, a doctor can carefully remove or correct problems with target tissues or materials.

Another device embodying the invention that can be used to determine tissue characteristics is shown, in longitudinal cross-section, in Figure 5. The instrument 110 includes a cylindrical outer housing 112 with a circular end cap 120 configured to abut the target tissue. A rotating cylindrical inner core 114 is mounted in the outer housing 112. A bundle of optical fibers 116 are located inside the inner core 114.

The optical fibers 116 pass down the length of the inner core 114 and are arranged in a specific pattern at the end adjacent the end cap 120 of the outer housing 112. The end of the inner core 114 adjacent the end cap 120 is mounted within the outer housing 112 with a rotating bearing 122. The end cap 120 is at least partially transparent or transmissive so that electromagnetic radiation can pass from the optical fibers, through the end cap, to illuminate a target tissue adjacent the end cap 120. Light scattered from or generated by the target tissue would then pass back through the end cap 120 and back down the optical fibers 116.

The inner core 114 is also mounted inside the outer housing 112 by a detent mechanism 118. The detent mechanism is intended to support the inner core 114, and ensure that the inner core is rotatable within the outer housing 112 by predetermined angular amounts.

A cross sectional view of an embodiment of the instrument, taken along section line 10-10 of Figure 5, is shown in Figure 6A. The inner core 114 is supported within the outer housing 112 by the detent mechanism. In this

embodiment, the detent mechanism includes two mounts 134 with spring loaded fingers 136 that are biased away from the inner core 114. The detent mechanism also includes four stoppers 130, each of which has a central depression 132. The spring loaded fingers 136 are configured to engage the central depressions 132 of the stoppers 130 to cause the rotatable inner core to come to rest at predetermined angular rotational positions. In the embodiment shown in Figure 6A, four stoppers are provided in the inner surface of the outer housing 112. Thus, the inner core 114 will be rotatable in increments of approximately 90°. In alternate embodiments similar to the one shown in Figure 6A, four mounts 134, each having its own spring loaded finger 136, could be attached to the inner core 114. The provision of four such mounts would serve to keep the inner core 114 better centered inside the outer housing 112.

An alternate embodiment of the detent mechanism is shown in Figure 6B. In this embodiment, six stoppers 130 are spaced around the inside of the outer housing 112. Three mounts 134, each having its own spring loaded finger 136, are mounted on the inner core 114. The three mounts 134 are spaced around the exterior of the inner core 114 approximately 120° apart. This embodiment will allow the inner core to be rotated to predetermined positions in increments of 60°. In addition, the location of the three mounts, 120° apart, helps to keep the inner core 114 supported in the center of the outer housing 112.

With reference to Figure 5, the ends of the optical fibers may be mounted on a circular end plate 121 that holds the optical fibers in a predetermined pattern. The circular end plate 121 would be rigidly attached to the end of the cylindrical inner core 114. In addition, an index matching agent 123 may be located between the end plate 121 and the end cap 120 on the outer housing 112. The index matching agent 123 can serve as both an optical index matching agent, and as a lubricant to allow free rotation of the end plate 121 relative to the end cap 120.

A diagram showing how the optical fibers are positioned on the face of an embodiment of the instrument is shown in Figure 7A. The face of the instrument, which would be the end cap 120 of the device shown in Figure 5, is indicated by reference number 140 in Figure 7A. The black circles 142 represent the locations of optical fibers behind the end cap 120. The hollow circles 144 represent the positions that the optical fibers will move to if the inner core 114 of the instrument is rotated approximately 90°. Thus, each of the circles represent positions that can be interrogated with the optical fibers.

In some embodiments of the device, a single optical fiber will be located at each of the positions shown by the black circles 142 in Figure 7A. In this instance, excitation light would travel down the fiber and be emitted at each interrogation position indicated by a black circle 142. Light scattered from or produced by the target tissue would travel back up the same fibers to a detector or detector array, such as detector 56 shown in Figure 1.

In alternate embodiments, pairs of optical fibers could be located at each position indicated by the black circles 142A, 142B, as shown in Figure 7B. In the alternate embodiments, one optical fiber of each pair would conduct excitation light to the target tissue, and the second optical fiber of each pair would conduct light scattered from or generated by the target tissue to a detector. In still other alternate embodiments, multiple fibers for carrying excitation light and/or multiple fibers for carrying light scattered from or generated by the target tissue could be located at each interrogation position indicated by a black circles 142A, 142B, 142C, 142D to allow simultaneous detection of, for example, both fluorescence and reflectance, as shown in Figure 7C. In this latter case, the optical fibers could be arranged to focus on the same point of subject tissue, as shown in Figure 7D.

To use an instrument having the optical fiber pattern shown in Figure 7A, the instrument would first be positioned so that the end cap 120 is adjacent the target tissue. The end cap 120 may be in contact with the target tissue, or it might

be spaced from the surface of the target tissue. Also, an index matching material may be interposed between the end cap 120 and the target tissue. Then, the optical fibers would be used during a first measurement cycle to simultaneously measure tissue characteristics at each of the interrogation positions in Figure 7A having a black circle 142. The tissue characteristics could be measured using any of the measurement techniques discussed above. Then, the inner core 114 would be rotated approximately 90° within the outer housing 112, and the optical fibers would be used during a second measurement cycle to simultaneously measure tissue characteristics at each of the interrogation positions in Figure 7 having a hollow circle 144.

The instrument may include markings (not shown) on the end cap 120 or elsewhere, which acts as a locator tool to allow a user to determine how many rotations have been made, and thus how much of the tissue has been analyzed.

Constructing an instrument as shown in Figures 5, 6A or 6B, and having any of the optical fiber patterns shown in Figures 7A-7D, may have certain advantages. For example, constructing an instrument in this manner allows the instrument to interrogate many more points in the target tissue than would have been possible if the inner core did not rotate. The ability to rotate the inner core 114, and take a second series of measurements at different locations on the target tissue, essentially increases the resolution of the device.

In addition, when a large number of optical fibers are packed into the tissue contacting face of an instrument, cross-talk between the optical fibers can occur. The cross-talk can occur when excitation light from one interrogation position scatters from the target tissue and enters an adjacent interrogation position. Cross-talk can also occur if excitation light from a first interrogation position travels through the target tissue and enters an adjacent interrogation position. One of the easiest ways to reduce or eliminate cross-talk is to space the interrogation positions

farther apart. However, increasing the spacing between interrogation positions will reduce the resolution of the device.

An instrument embodying the invention, with a rotatable inner core, allows the interrogation positions during any single measurement cycle to be spaced far enough apart to reduce or substantially eliminate cross-talk. Because multiple measurement cycles are used, the device is able to obtain excellent resolution. Thus, good resolution is obtained without the negative impact to sensitivity or selectivity caused by cross-talk. In addition, fewer optical fibers and fewer corresponding detectors are required to obtain a given resolution.

In addition, the ability to obtain a plurality of tissue measurements simultaneously from positions spaced across the entire target tissue has other benefits. If the instrument is intended to detect dysplastic growths or other tissue maladies, the target tissue area interrogated by the instrument is likely to have both normal tissue, and diseased tissue. As noted above, tissue characteristics can vary significantly from person to person, and the tissue characteristics can vary significantly over relatively short periods of time. For these reasons, one way to determine the locations of diseased areas is to establish a baseline for normal tissue, then compare the measurement results for each interrogation point to the baseline measurement. The easiest way to determine the location of a diseased area is to simply look for a measurement aberration or variance.

Because tissue characteristics can change relatively quickly, in order to establish accurate, clearly defined variances between tissue characteristics, it is desirable to take a plurality of readings simultaneously over as large an area as possible. In some embodiments of the invention, this could include taking fluorescence measurements at a plurality of interrogation points, and then subsequently taking reflectance measurements, at the same plurality of interrogation points. Alternately, the fluorescence and reflectance measurements could be taken simultaneously.

Ideally, all measurements should be conducted during the same time period. However, in an embodiment where different measurements are taken at different times, the apparatus and method could be configured to conduct all the measurements within a critical time window. The critical time window is defined as the maximum duration of time between two spectroscopic measurements which yields the benefits described herein. The critical timing window may vary depending on a variety of factors including those described below. In some embodiments of the invention, a critical timing window between subsequent measurements was less than approximately 0.25 seconds. This critical timing window gave favorable results.

There are several effects which make it desirable to conduct fluorescent and reflectance measurements of the interrogated points either simultaneously, or as nearly simultaneously as possible. First, changes in blood pressure, which occur during each heart beat cycle can have a large affect on blood content in the tissue. Because blood strongly absorbs certain wavelengths of light, the varying amount of blood present at an interrogated point during different parts of the heart beat cycle can cause significantly varying measurement results.

To eliminate this potential error source, both fluorescent and reflectance measurements should be taken within a small enough time window that the blood content remains the same. Time periods of less than approximately 0.25 seconds should be sufficient. Another way to eliminate the potential error is to take multiple measurements of the same interrogation point during different portions of the heart beat cycle, then average the results.

Another factor to consider is patient movement. If the patient moves, even slightly, during a measurement cycle, the contact pressure between the measurement instrument and the interrogated tissue can change. This can also affect the measurement results. Thus, obtaining measurements simultaneously, or as

nearly simultaneously as possible, also helps to prevent measurement errors caused by patient movement.

Also, because tissue tumors can be as small as approximately 1 mm, the resolution of the device is preferably approximately 1 mm. In other words, to obtain the requisite resolution, the spacing between interrogation positions should be approximately 1 mm. Unfortunately, when the interrogation positions are approximately 1 mm apart during a single measurement cycle, significant cross-talk can occur, and the accuracy of the measurement results is poor.

An instrument embodying the invention allows the interrogation positions to be spaced sufficiently far apart to essentially eliminate cross-talk, while still obtaining the requisite 1 mm resolution. Although not all measurements are obtained at exactly the same time, during each measurement cycle, simultaneous measurements are made at positions spaced across the entire target tissue, which should include both normal and diseased areas. Thus, the results from each measurement cycle can be used to detect variances in tissue characteristics that help to localize diseased areas. For these reasons, an instrument embodying the invention balances the competing design requirements of resolution, elimination of cross-talk, and the desire to make all measurements simultaneously to ensure that time-varying tissue characteristics are taken into account.

A second arrangement for the optical fibers of a device as shown in Figure 5 is depicted in Figure 8. In this embodiment, the interrogation positions are arranged in a hexagonal honeycomb pattern. The black circles 142 indicate the positions that would be occupied by optical fibers during a first measurement cycle, and the hollow circles 144 indicate positions that would be occupied by the optical fibers during a second measurement cycle after the inner core 112 has been rotated by approximately 60°. This pattern achieves maximum spacing between adjacent interrogation positions during each measurement cycle, and essentially doubles the resolution of the instrument.

A third arrangement for the optical fibers of a device shown in Figure 5 is depicted in Figure 9. In this embodiment, the optical fibers are again arranged according to a hexagonal honeycomb pattern. However, far fewer optical fibers are used in this embodiment. This third embodiment is intended for use in a measurement process that calls for six measurement cycles. The inner core of the device would be rotated approximately 60° between each measurement cycle. Over the course of the six measurement cycles, the device would ultimately interrogate all the black circled 142 and hollow circled 144 interrogation positions shown in Figure 9. This embodiment allows for even greater separation distances between the interrogation positions during a single measurement cycle (to reduce or substantially eliminate cross-talk), while still achieving excellent measurement resolution. In addition, far fewer optical fibers and corresponding detectors would be required to achieve a given measurement resolution.

Experimental studies were conducted by the applicants to determine the spacing between interrogation positions that is needed to substantially eliminate cross-talk. The studies were conducted using a pair of optical fibers at each interrogation position, wherein one fiber in each pair provides excitation light, and the other fiber in each pair is used to detect light. The excitation optical fibers had a diameter of approximately 200 μm, the detection fibers had a diameter of approximately 100 μm. Measurements were made on optical reference standards, and tissue. Under these conditions, it was necessary to space the interrogation positions approximately 3 mm apart to substantially eliminate cross-talk. Thus, if an instrument were not designed as described above, so that the inner core can rotate the interrogation positions to different locations on the target tissue, the device would only be capable of achieving a resolution of approximately 3 mm.

One embodiment of the invention utilizes an optical fiber pattern similar to the one shown in Figure 9. Thus, the device is designed to conduct six measurement cycles to complete all measurements within the target tissue. The

inner core 114 is rotated 60° between each measurement cycle. This embodiment utilizes optical fiber pairs at each interrogation position. Each optical fiber pair includes an excitation fiber having an approximately 200 μm diameter, and a detection optical fiber having an approximately 100 μm diameter. The arrangement of the optical fibers allows the interrogation positions to be spaced approximately 3.0-3.5 mm apart, while still achieving a resolution of approximately 1 mm.

To determine the locations of diseased areas within a target tissue it is necessary to take measurements at a plurality of different locations in the target tissue spaced in at least two dimensions. Each measurement may require multiple excitation wavelengths, and detection of multiple wavelengths of scattered or generated light. Thus, the measurements involve three measurement dimensions, two dimensions for the area of the target tissue, and a third dimension comprising the spectral information. A device capable of conducting measurements in these three dimensions is shown in Figure10.

The instrument includes a light source 20, and a filter assembly 22. A plurality of excitation optical fibers 116a lead from the filter assembly 22 to the target tissue 50. A plurality of detection fibers 116b lead away from the target tissue 50. The excitation optical fibers 116a and the detection optical fibers 116b are arranged in pairs as described above.

The light source 20 and filter assembly 22 allow specific wavelengths of light to be used to illuminate the target tissue 50 via the excitation optical fibers 116a. The filter assembly 22 could be a single band pass optical filter, or multiple optical filters that can be selectively placed between the light source 20 and the excitation optical fibers 116a. Alternatively, the light source 20 and filter assembly 22 could be replaced with a wavelength tunable light source. In yet other alternate embodiments, a plurality of light sources, such as lasers, could be used to selectively output specific wavelengths or wavelength bands of excitation light. Other sources may also be appropriate.

The detection fibers lead to an optical system 55. The light from the detection fibers 116b passes through the optical system and into a detector array 56. The detector array may comprise a plurality of photosensitive detectors, or a plurality of spectrophotometers. The detector array 56 is preferably able to obtain measurement results for each of the detection fibers 116b simultaneously.

The optical system 55 can include a plurality of optical filters that allow the detector array 56 to determine the intensity of light at certain predetermined wavelengths. In one embodiment, the detector array would be a two dimensional array of photosensitive detectors, such as a charge coupled device (CCD). The optical system would comprise a spectrograph that is configured to separate the light from each detection optical fiber 116b into a plurality of different wavelengths, and to focus the different wavelengths across a line of pixels on the CCD. Thus, each line of pixels on the CCD would correspond to a single detection fiber. The intensities of the different wavelengths of light carried by a single detection fiber 116b could be determined based on the outputs of a line pixels of the CCD. The greater the output of a particular pixel, the greater the intensity at a particular wavelength.

This embodiment is able to achieve excellent flexibility. Because all wavelengths of light are always detected, the instrument software can simply select the pixels of interest for each measurement, and thereby determine the intensity at particular wavelengths. During a first measurement, certain pixels representative of fluorescent characteristics could be examined. During a subsequent measurement, different pixels representative of scattering characteristics could be examined. Also, the device could be essentially re-configured to take completely different measurements by simply changing the control software. Thus, a single device could be used for a wide variety of different kinds of measurements.

In some methods embodying the invention, one of the structures described above would be used to conduct a series of measurements cycles. Where the

embodiment having the rotatable core is employed, the inner core of the device would be rotated between measurement cycles. Once all measurements of a measurement cycle are completed, the inner core would be rotated, and additional measurement cycles would be conducted.

In the methods, however, measurements are conducted using two or more spectroscopic methods during each measurement cycle. For instance, during a single measurement cycle the device may conduct a measurement of fluorescent characteristics, and a measurement of reflectance characteristics. However, other measurements and combinations of spectroscopic methods may also be appropriate. Then, the fluorescence and reflectance measurements can be compared and analyzed to decouple the effects due to biochemical and morphological tissue changes to provide for a more accurate diagnosis of the tissue's conditions.

As previously discussed, the measurements can be taken over substantially the entire surface area of the subject tissue, simultaneously or in intervals, and the results analyzed. Alternatively, the subject tissue can be divided into field areas to create a field pattern. Dividing the subject tissue into field areas allows analysis of particular areas of the tissue, for example, particular areas of the tissue where changes are likely to occur.

For example, the apparatus of Figure 1 could further include a field area adjusting unit 560 and field area processing unit 570, as shown in Figure 11A. The field area adjusting unit 560 would divide the target tissue into a plurality of field areas 580, as shown in Figures 11B-11D, to create a field pattern 500. The field areas 580 could be any desired shape and size (see, for example, the different sized and shaped field areas shown in Figures 11B-11D). Further, the divisions could be based on visual inspection of the target tissue, or on results of previous testing performed on the target tissue, and could be preprogramed into the apparatus, or input by a user. Measurements would then be taken by the detector 54 at each of a plurality of interrogation points 542 within the respective field area and the field area

processing unit 570 would then analyze the measurements for each of the respective field areas 580. The field area processing unit 570 could further compare the results for each respective field area 580 to the results for other field areas 580.

Figure 11B and 11C show 4 and 8 "pie-shaped" field areas, respectively. In each case, after measurements were taken by the detector 54 at each of a plurality of interrogation points 542 within the respective field areas and the results analyzed by the field area processing unit 570 for each of the respective field areas 580, the field area adjusting unit 560 could reset the field areas 580 by rotating the field area to group different sets of interrogation points (see arrow in Figure 11C), or could set field areas having a different size and shape, such as the field areas shown in Figure 11D. As shown in Figure 11 D, these field areas do not need to be identical in size and/or shape.

Alternatively, the field area adjusting unit 560 and field area processing unit 570 could be incorporated into the processor 44 and the divisions could be preprogramed into the processor or accompanying software.

Figure 12 shows steps of a method according to the invention. In a first step S1000, a target tissue is illuminated with electromagnetic radiation at predetermined wavelengths, one wavelength for detecting fluorescence characteristics and one wavelength for detecting reflectance characteristics. In a second step S1010, the detector 56, utilizing one of the optical fiber arrangements discussed above, detects returned electromagnetic radiation. In step S1020, the fluorescence and reflectance intensities are calculated, and in step S1030, the fluorescence and reflectance intensities are compared and analyzed using a method discussed below. In step S1040, the tissue characteristics are determined.

The deconvolution, or decoupling can be carried out in a variety of ways as described below. Any or all of the discriminant parameters can be combined together in order to improve the overall discrimination.

1. Using a linear combination of fluorescence and reflectance measured intensities as the discriminant parameter.

$$P = aF_m + bR_x + cR_m \qquad \text{Equation 1}$$

Where x is the fluorescence excitation wavelength, m is the fluorescence emission wavelength, F is the fluorescence intensity and R is the reflectance intensity. The factors a, b and c are weighing factors that are empirically selected to give the best discrimination.

2. Using a linear combination for fluorescence and reflectance ratios as the discriminant parameter.

$$P = a\frac{F_m}{R_m} + b\frac{F_m}{R_x} \qquad \text{Equation 2}$$

3. Using a linear combination for fluorescence and reflectance ratios at multiple fluorescence emission wavelengths as the discriminant parameter.

$$P = a\frac{F_{1m}}{F_{2m}} + b\frac{R_{1m}}{R_{1x}} + c\frac{R_{2m}}{R_{2x}} \qquad \text{Equation 3}$$

Where 1m and 2m are two distinct fluorescence emission wavelengths, 1x and 2x are the corresponding excitation wavelengths, F is the fluorescence intensity and R is the reflectance intensity. The factors a, b and c are weighing factors that are empirically selected to give the best discrimination.

4. Using quantum yield (also known as quantum efficiency) measurement as the discriminant parameter. The quantum yield defines the true fluorescence yield in terms of the number of fluorescence photons generated by the fluorophore per photon of light absorbed.

$$P = \frac{aF_m}{1 - bR_x} \qquad \text{Equation 4}$$

The fluorescence and reflectance intensities are corrected for background light and normalized to the intensities measured off a calibration target. The factors a and b are weighing factors that are empirically selected to give the best discrimination.

5. Blood has broadband absorbance with three distinct visible peaks at around 410 nm, 545 nm and 575 nm. On the one hand, blood absorbance changes from increased vascularization in cancer tissue, and is an important marker for disease. On the other hand, blood absorbance related artifacts occur in the measured spectra from local bleeding and inflammation. The spectral discriminant factor described above must therefore be corrected for blood absorbance. This can either be done by normalizing the discriminant factor to blood reflectance.

$$P_{corr} = \frac{P}{R_{blood}} \qquad \text{Equation 5}$$

Or by subtracting the blood reflectance.

$$P_{corr} = P - d.R_{blood} \qquad \text{Equation 6}$$

Where d is an empirical correction factor and R_{blood} is the reflectance of blood at an empirically selected wavelength.

6. Alternatively the intensity set, F_m, R_m and R_x, where is selected for each fluorophore are collectively modulated against the pathology results in a principle component analysis or a logistic regression. These can then form the basis of pattern recognition techniques, such as, for example, classification and regression trees (CART), as taught by L. Brieman, *et al.* in Classification and Regression Trees, Monterey CA: Wadsworth & Brooks/Cole, 1984, which is hereby incorporated by reference, normal networks and hybrids thereof.

The techniques according to the invention are designed to discriminate normal tissue from various dysplastic tissue stages based on spectroscopic data alone. Additional factors, such, as for example, patient age, menopausal status, menstrual state, previous history of disease can be added to the spectroscopic input in achieving better discrimination.

In each of the embodiments described above, in which a plurality of measurement cycles are conducted on a target tissue, and an inner core having optical fibers arranged in a predetermined pattern is rotated between measurement cycles to make a plurality of measurements on a target tissue, alternate embodiments could use some other movement mechanism other than a rotating one. The invention encompasses other types of movement or translational devices that allow a plurality of measurements to be taken on a target tissue with a limited number of detectors that are spaced far enough apart to avoid cross-talk. Also, as previously discussed, the measurements could be taken over the entire area of the subject tissue simultaneously, or the target tissue could be divided into field areas and measurements could be taken in each field area.

Further, the apparatus and methods embodying the invention make it possible to conduct in vivo measurements of tissues on the inside of body passages or lumens. An endoscope embodying the invention can be inserted into a natural body lumen of a human or animal to search for the presence of dysplastic or diseased tissue. This means that no surgery would be required to locate and examine tissues inside the body of the human or animal under study.

The use together of fluorescence measurements along with reflectance measurements provides a more accurate determination of target tissue characteristics than one of the measurements alone.

The techniques described above can be used to map the conditions of an area of target tissue. For instance, the above-described techniques can be used to determine a condition of a target tissue adjacent a distal end of a measuring device. The measuring device could then be moved adjacent a different portion of the target tissue, and the measurements could be repeated. This process could be repeated numerous times to determine the conditions of different portions of a target tissue area. The determined conditions could then be used to create a map of the target tissue area, which could be printed or displayed on a monitor.

One of the most difficult problems with in vivo tissue diagnostics and disease measurement is the biological diversity of normal tissue properties between different patients, or even within the same patient. Furthermore, this diversity is time variant both in the long term and in the short term. Long term variations may be due to patient age, hormonal milieu, metabolism, mucosal viscosity, and circulatory and nervous system differences. Short term variations may be from blood perfusion changes due to heart beat, physical movement, local temperature changes etc.

Because of the variability of tissue characteristics, to accurately determine whether a target tissue is diseased, one needs to compare measurements of the target tissue to measurements of normal tissues from the same patient. The measurements of the known normal tissue should be made concurrently or simultaneously with the measurements of the target tissue. The normal tissue measurements then serve as a baseline for normalcy, variations from which may be interpreted as disease. To arrive at a baseline measurement, a number of strategies can be used.

First, visual characteristics such as pigmentations (nevi) in skin, or polyps in the colon, can be used to identify potentially abnormal regions. Normalized or averaged spectra of multiple regions surrounding these potentially abnormal, visually distinct regions can be used to establish baseline measurements. The baseline measurements can then be compared to measurements taken on the abnormal, visually distinct regions.

Measurements of normal and abnormal regions based on visual characteristics could be automated using imaging capabilities of the measurement device itself.

In an alternate strategy, measurements can be taken on spaced apart regions along a portion of a lumen or tissue. The spacing between the regions would be dependent on the type of tissue being diagnosed. Then, differentials between individual measurements taken at different regions would be calculated. If differentials are greater than a preset amount, the tissue between the excessively

high differentials would be diagnosed as diseased. In yet another alternate strategy, a gradient in spectral response as one moves away from a visually suspicious site could also be used as a marker for disease. This is easily automated and can be implemented effectively in any imaging modality.

In addition, pattern recognition algorithms (e.g. neural nets) could also be used to analyze differences in readings taken from various sites in the same patient or from multiple readings from different patients.

Figure 13 is a Receiver Operating Characteristic (ROC) plot of sensitivity versus one minus specificity comparing results obtained according to the invention to results obtained using pap smear testing and the line of chance. This graph exhibits that testing using the invention is much more successful at differentiating high grade from low grade lesions, than, for example, pap smear testing.

A similar ROC plot is shown in Figure 14. The plot indicates that devices embodying the invention, as described above, are very good at distinguishing dysplastic tissue from normal tissue.

Results obtained according to the invention can be mapped using false color maps to identify abnormal tissue. Figures 15A and 15B show, respectively, the mapping of the results from two patients using false color mapping. The map on the left shows low grade lesions, the map on the right shows high grade lesions. Individual pixels on the map correspond to an individual measurement site on a patient's cervix. The results of the fluorescence and reflectance measurements made at the site are used to assign a color or darkness to the pixel.

The variations in color or darkness in Figure 15A indicate the existence and location of low grade lesions. The results in Figure 15A are consistent with the pap smear and pathology testing results for the patient, which both detected low grade lesions, while examination of the patient's cervix using a colposcope showed no lesions.

The variations in color or darkness in Figure 15B represent the existence of high grade lesions, the darker color indicating a more diseased tissue state. The results in Figure 15B are consistent with colposcopy and pathology testing results for the patient, which all detected high grade lesions, while pap smear testing showed reactive changes.

The invention could also be implemented using a multi-modal hyperspectral imaging (MHI) camera system. The MHI camera system would collect measurements over a specified field according to the invention and then create an image according to the collected data. Exemplary images produced according to the invention using an MHI camera system are shown in Figures 16A and 16B. In Figure 16A, the lighter portions of the image are indicative of high grade squamous intraepithelial lesions (SIL), while in Figure 16B, there is no indication of abnormal tissue condition.

Although it is premature to draw definitive conclusions regarding this small data set, the result are encouraging. There were high grade lesions misclassified by both Pap tests and colposcopy which could be discriminated by the spectroscopic methods of the invention. Moreover, the results of these preliminary cases are consistent with known biologic phenomena and field effects due to carcinogenesis. Of note is that both fluorescence and reflectance measurements provide discriminative information.

Having looked at overall means, standard deviation and coefficient of variation at individual wavelengths, spatial and spectral information can then be exploited. Those spectra measured from points on the tissue for which histopathology is available (e.g., at/near a biopsy site) can be examined specifically by category, for example, normal versus abnormal. To further utilize spectral information, the preferred method involves taking various intensity ratios at the key wavelengths discussed above. Beyond that approach,

advanced statistical analysis techniques (for example, principal component analysis, Bayesian Classification, Classification Trees, Artificial Neural Networks,) may be used to help to identify other wavelengths which can be effective for discriminating and modeling a pattern recognition.

The foregoing embodiments are merely exemplary and are not to be construed as limiting the invention. The present teaching can be readily applied to other types of apparatuses. The description of the invention is intended to be illustrative, and not to limit the scope of the claims. Many alternatives, modifications, and variations will be apparent to those skilled in the art.

WHAT IS CLAIMED IS:

1. A method for diagnosing a condition of a target tissue, comprising the steps of:

a.) irradiating a target tissue;

b.) sensing a returned radiation;

c.) determining characteristics of the returned radiation using at least two spectroscopic methods;

d.) combining the characteristics determined by the at least two spectroscopic methods; and

e.) determining a condition of the target tissue based on the combined determined characteristics.

2. The system of claim 1, wherein the at least two spectroscopic methods comprise fluorescence measurements and scattering or reflectance measurements.

3. The method of claim 1, wherein the at least two spectroscopic methods are selected from the group consisting of absorption measurements, scattering measurements, reflection measurements, polarization anisotropic measurements, steady state fluorescence measurements, and time resolved fluorescence measurements.

4. The method of claim 3, wherein the time resolved fluorescence measurements comprise at least one of phase modulation techniques, polarization anisotropic techniques and techniques that directly monitor the decay profile of fluorescent emissions.

5. The method of claim 1, wherein step a.) comprises illuminating the target tissue with excitation electromagnetic radiation, and wherein step b.) comprises simultaneously sensing electromagnetic radiation emitted from the target tissue in response to the excitation electromagnetic radiation and excitation electromagnetic radiation that is scattered from the target tissue.

6. The method of claim 5, wherein step c.) comprises making intensity based measurements on both said electromagnetic radiation emitted from the target tissue in response to the excitation electromagnetic radiation and said excitation electromagnetic radiation that is scattered from the target tissue.

7. The method of claim 1, wherein step a.) comprises illuminating the target tissue with excitation electromagnetic radiation, and wherein step b.) comprises sensing electromagnetic radiation emitted from the target tissue in response to the excitation electromagnetic radiation and then subsequently sensing excitation electromagnetic radiation that is scattered from the target tissue.

8. The method according to claim 7, wherein a critical timing window, which is defined as the time period between sensing electromagnetic radiation emitted from the target tissue in response to the excitation electromagnetic radiation and subsequently sensing excitation electromagnetic radiation that is scattered from the target tissue, is not greater than approximately 0.25 seconds.

9. The method of claim 7, wherein step c.) comprises making intensity based measurements on both said electromagnetic radiation emitted

from the target tissue in response to the excitation electromagnetic radiation and said excitation electromagnetic radiation that is scattered from the target tissue.

10. The method of claim 1, wherein step b.) comprises sensing, approximately simultaneously, radiation returned from a plurality of interrogation points distributed over the target tissue.

11. The method according to claim 10, further comprising a step of dividing the target tissue into a first set of field areas, wherein step c.) comprises determining characteristics of the returned radiation in each of said first set of field areas using at least two spectroscopic methods, step d.) comprises combining the characteristics determined by the at least two spectroscopic methods for each of said first set of field areas and step e.) comprises determining a condition of the target tissue by comparing the combined determined characteristics of each of said first set of field areas.

12. The method according to claim 11, further comprising a step of identifying visual characteristics of the target tissue, wherein the field areas are selected based on the identified visual characteristics of the target tissue.

13. The method according to claim 11, wherein the field areas are selected based on previously identified characteristics of the target tissue.

14. The method according to claim 13, wherein the previously identified characteristics of the target tissue comprise characteristics of the target tissue identified through previous testing of the target tissue using at least one of cytology, colposcopy and histopathology.

15. The method of claim 11, further comprising, after determining a condition of the target tissue by comparing the combined determined characteristics of each of said first set of field areas, re-dividing the target tissue into a second set of field areas, different from said first set of field areas and the determining characteristics of the returned radiation in each of said second set of field areas using at least two spectroscopic methods, combining the characteristics determined by the at least two spectroscopic methods for each of said second set of field areas and determining a condition of the target tissue by comparing the combined determined characteristics of each of said second set of field areas.

16. The method of claim 10, wherein the method is performed using an apparatus comprising an irradiation source, a detector and a processor, wherein the step of sensing radiation returned from a plurality of interrogation points comprises the steps of:

sensing radiation returned from the target tissue from a first subset of the plurality of interrogation points;

moving at least one of the apparatus and the tissue;

sensing radiation returned from the target tissue from a second subset of the plurality of interrogation points;

again moving at least one of the apparatus and the tissue; and

continuing this process until sensing has been peformed at all of the plurality of interrogation points.

17. The method of claim 1, further comprising a step of generating a map of conditions of different portions of the target tissue based on the combined determined characteristics.

18. The method of claim 1, further comprising a step of conducting a pattern recognition process to determine whether a pattern of conditions exists within the target tissue.

19. A system for determining a condition of a target biological tissue, comprising:

a source for providing excitation radiation;

a device that couples the excitation radiation to a target tissue;

a device that senses radiation returned from the target tissue;

a processor configured to determine characteristics of the returned radiation using at least two spectroscopic methods, wherein the processor combines the characteristics determined by each of the at least two spectroscopic methods in order to decouple biochemical changes from morphological changes in the target tissue and determines a condition of the target tissue based on the combined determined characteristics.

20. The system of claim 19, wherein the at least two spectroscopic methods comprise fluorescence measurement methods and scattering or reflectance measurement methods.

21. The system of claim 19, wherein the at least two spectroscopic methods are selected from the group consisting of absorption measurements, scattering measurements, reflectance measurements, polarization anisotropy measurements, steady state fluorescence measurements and time resolved fluorescence measurements.

22. The system of claim 19, wherein the device that senses returned radiation is configured to simultaneously sense fluorescent radiation emitted by

endogenous fluorophores in response to the excitation radiation and excitation radiation that is scattered from the target tissue.

23. The system of claim 22, wherein the processor makes intensity based measurements on both said fluorescent radiation emitted by endogenous fluorophores in response to the excitation radiation and said excitation radiation that is scattered from the target tissue.

24. The system of claim 19, wherein the device that senses radiation is configured to first sense fluorescent radiation emitted by fluorophores in response to the excitation radiation and then subsequently sense excitation radiation that is scattered from the target tissue.

25. The system according to claim 24, wherein a critical timing window, which is defined as the time period between sensing radiation emitted from the target tissue in response to the excitation radiation and subsequently sensing excitation radiation that is scattered from the target tissue, is not greater than approximately 0.25 seconds.

26. The system of claim 24, wherein the processor makes intensity based measurements on both said fluorescent radiation emitted by endogenous fluorophores in response to the excitation radiation and said excitation radiation that is scattered from the target tissue.

27. The system of claim 19, wherein the device that senses radiation is configured to sense radiation returned from a plurality of interrogation points distributed over the target tissue.

28. The system according to claim 27, wherein the processor divides the target tissue into a first set of field areas, determines characteristics of the returned radiation in each of said first set of field areas using said at least two spectroscopic methods, combines the characteristics determined by each of said at least two spectroscopic methods for each of said first set of field areas and determines a condition of the target tissue in each of said first set of field areas based on the combined determined characteristics of the respective field areas.

29. The system according to claim 28, wherein the target tissue is divided into field areas according to previously identified characteristics of the target tissue.

30. The system according to claim 29, wherein the previously identified characteristics of the target tissue are visually identified characteristics of the target tissue.

31. The system according to claim 29, wherein the previously identified characteristics of the target tissue are characteristics of the target tissue identified through previous testing of the target tissue using at least one of cytology, colposcopy and histopathology.

32. The system of claim 28, wherein the processor is further configured to, after the processor determines a condition of the target tissue in each of the first set of field areas based on the combined determined characteristics of the respective field areas, divide the target tissue into a second set of field areas, different from the first set of field areas; determine characteristics of the returned electromagnetic radiation in each of said second set of field areas using said at least two spectroscopic methods, combine the characteristics determined by each

of said at least two spectroscopic methods for each of said second set of field areas and determine a condition of the target tissue in each of the second set of field areas based on the combined determined characteristics of the respective field areas.

33. The system of claim 27, wherein the device that senses radiation is movable to a plurality of pre-determined positions and is configured to sense radiation returned from a subset of the plurality of interrogation points at each pre-determined position.

34. The system of claim 19, wherein the processor is also configured to conduct a pattern recognition process to determine whether a pattern of conditions exists within the target tissue.

35. The system of claim 19, wherein the processor is also configured to create a map of determined conditions of different portions of a target tissue.

36. A method for diagnosing dysplastic tissue, comprising:

irradiating a target tissue with radiation;

sensing radiation returned from the target tissue;

determining characteristics of the returned electromagnetic radiation using at least two spectroscopic methods, thereby decoupling biochemical changes from morphological changes in the target tissue occurring due to disease; and

determining a condition of the target tissue based the determined characteristics.

37. A system for determining a condition of a target biological tissue, comprising:

a radiation source for providing excitation radiation;

a device that couples the excitation radiation to a target tissue;

a device that senses radiation returned from the target tissue; and

a processor configured to determine characteristics of the returned radiation using at least two spectroscopic methods, thereby decoupling biochemical changes from morphological changes in the target tissue occurring due to disease and determine a condition of the target tissue based on the determined characteristics.

38. The method according to claim 1, wherein step c) comprises taking fluorescence and scattering or reflective measurements at two wavelengths at each of a plurality of interrogation points; step d) comprises determining a fluorescence ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points and averaging the fluorescence ratios and determining a scattering or reflectance ratio for the measurements taken at the two wavelengths at each of the plurality of interrogated points and averaging the scattering or reflectance ratios; and step e) comprises determining a condition of the target tissue based on the averaged fluorescence and scattering or reflectance ratios.

39. The method of claim 1, wherein step c) comprises taking fluorescence and scattering or reflectance measurements at two wavelengths at each of a plurality of interrogation points; step d) comprises determining a fluorescence ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points, averaging the fluorescence ratios, determining a coefficient of variation value for the averaged fluorescence ratio, determining a

scattering or reflectance ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points, averaging the scattering or reflectance ratios, determining a coefficient of variation value for the averaged scattering or reflectance ratio; and step e) comprises determining a condition of the target tissue based on the coefficient of variation values.

40. The method of claim 1, further comprising mapping the characteristics determined by the at least two spectroscopic methods using false color mapping, wherein step e) comprises identifying abnormal tissue using the false color map.

41. The method of claim 1, wherein a multi-modal hyperspectral imaging camera is used to implement the method.

42. The system according to claim 19, wherein the processor makes fluorescence and scattering or reflective measurements at two wavelengths at each of a plurality of interrogation points, determines a fluorescence ratio for the fluorescence measurements taken at the two wavelengths at each of the plurality of interrogation points and averages the fluorescence ratios, and determines a scattering or reflectance ratio for the scattering or reflectance measurements taken at the two wavelengths at each of the plurality of interrogation points and averages the scattering or reflectance ratios.

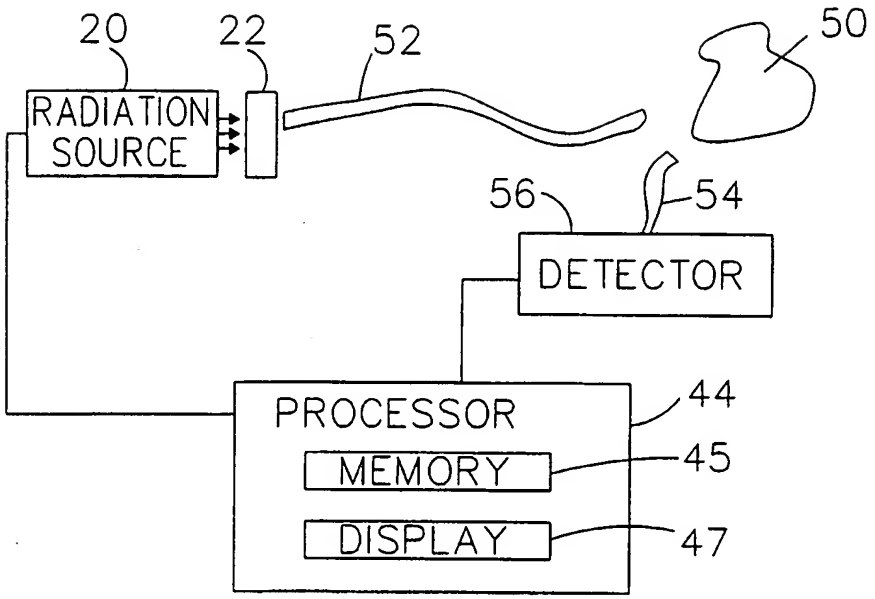
43. The system of claim 19, wherein the processor makes fluorescence and scattering or reflectance measurements at two wavelengths at each of a plurality of interrogation points, determines a fluorescence ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points, averages the fluorescence ratios, determines a coefficient of

variation value for the averaged fluorescence ratio, determines a scattering or reflectance ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points, averages the scattering or reflectance ratios and determines a coefficient of variation value for the averaged scattering or reflectance ratio.

44. The system of claim 43, wherein the processor determines the condition of the target tissue based upon the coefficient of variation values for the averaged fluorescence and scattering or reflectance ratios.

45. The method according to claim 1, wherein step c) comprises taking fluorescence and scattering or reflective measurements at two wavelengths at each of a plurality of interrogation points; step d) comprises determining a fluorescence to scattering or reflectance ratio for the measurements taken at the two wavelengths at each of the plurality of interrogation points and averaging the fluorescence to scattering or reflectance ratios; and step e) comprises determining a condition of the target tissue based on the averaged fluorescence to scattering or reflectance ratio.

46. The method according to claim 1, wherein step c) comprises taking fluorescence and scattering or reflective measurements at two wavelengths at each of a plurality of interrogation points; step d) comprises using one or more statistical method to analyze the fluorescence and scattering or reflective measurements; and step e) comprises determining a condition of the target tissue based on the results of the statistical analysis.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DETECTOR
PROCESSOR
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MEMORY
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DISPLAY
47

FIG. 1

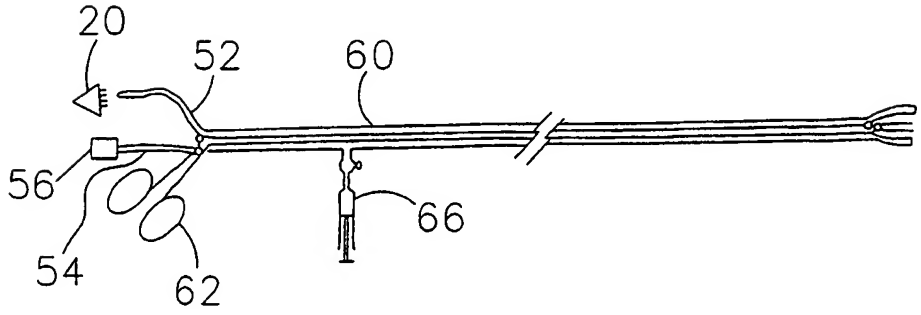
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FIG. 2

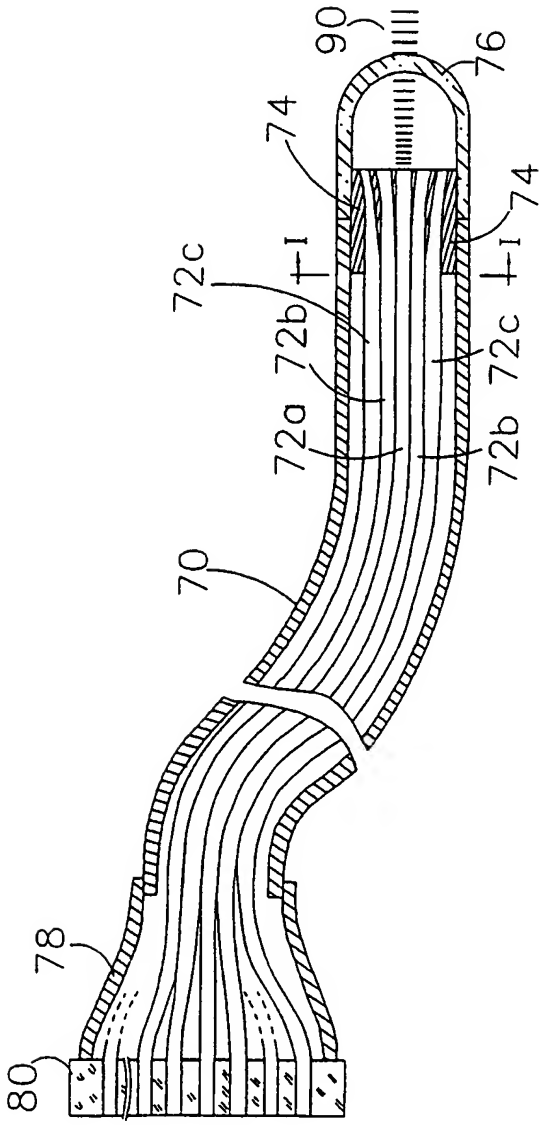
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FIG. 3A

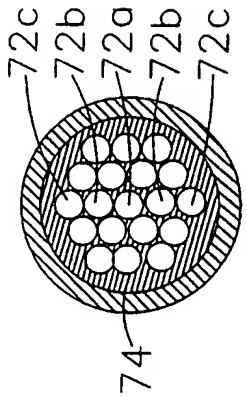
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FIG. 3B

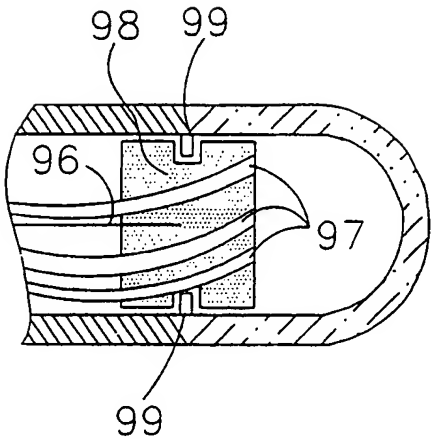


FIG. 4A

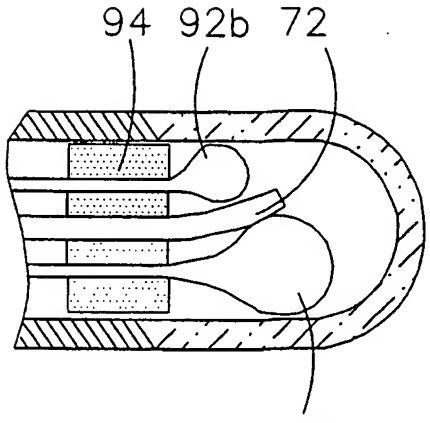


FIG. 4B

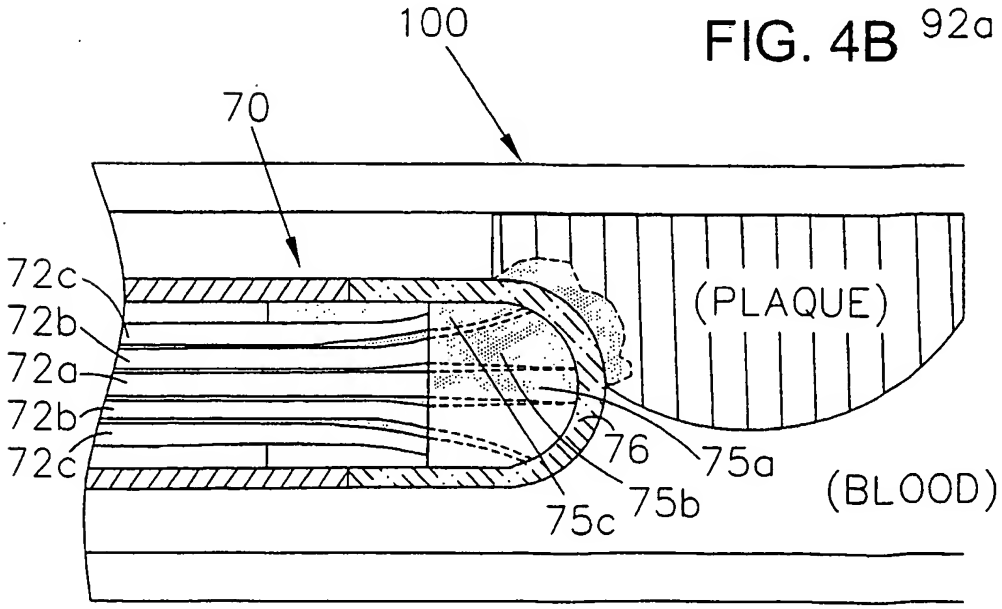


FIG. 4C

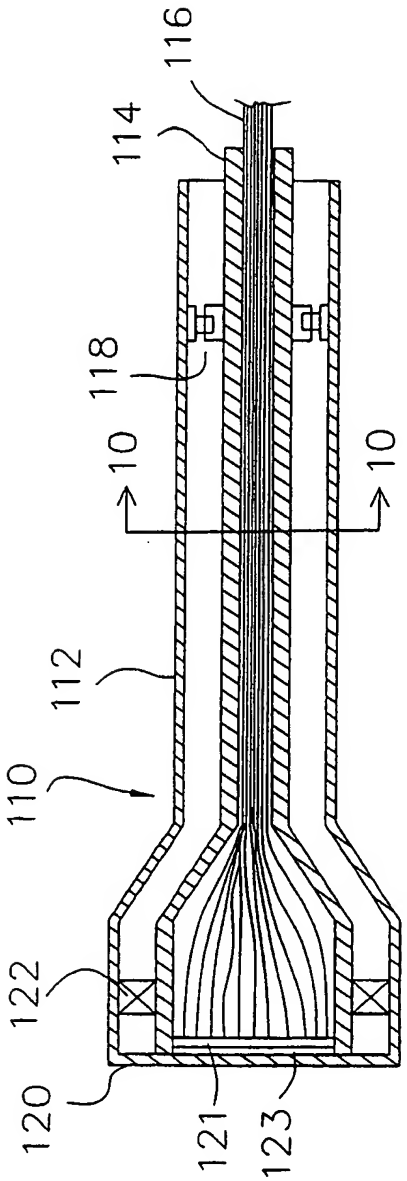


FIG. 5

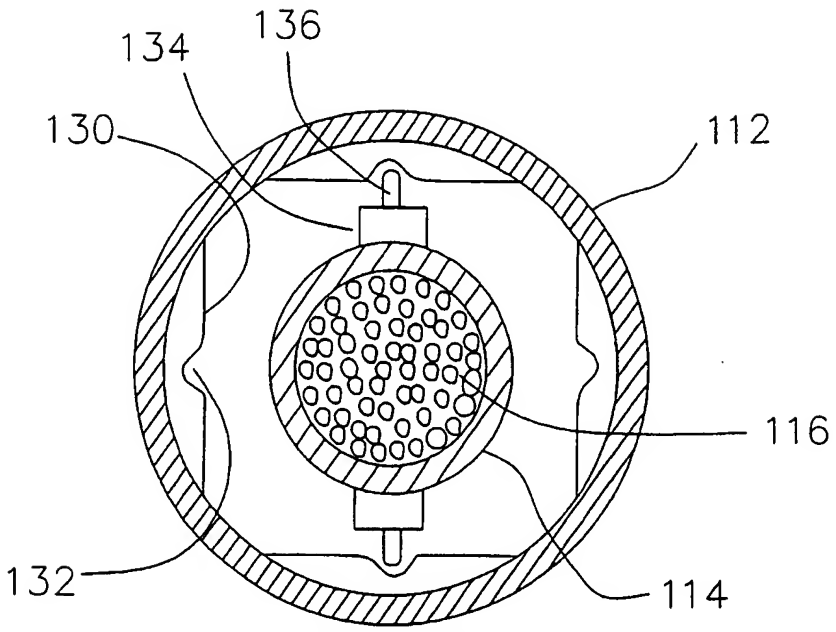


FIG. 6A

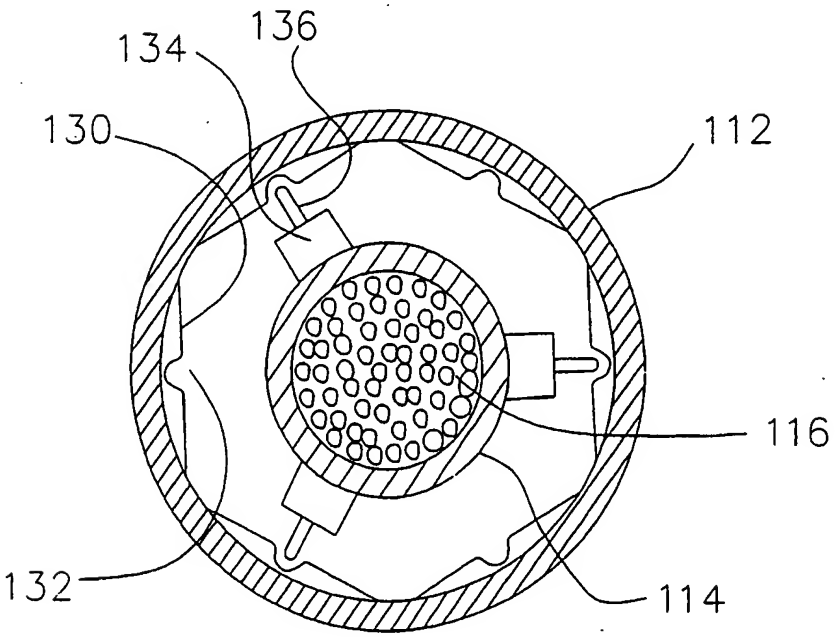


FIG. 6B

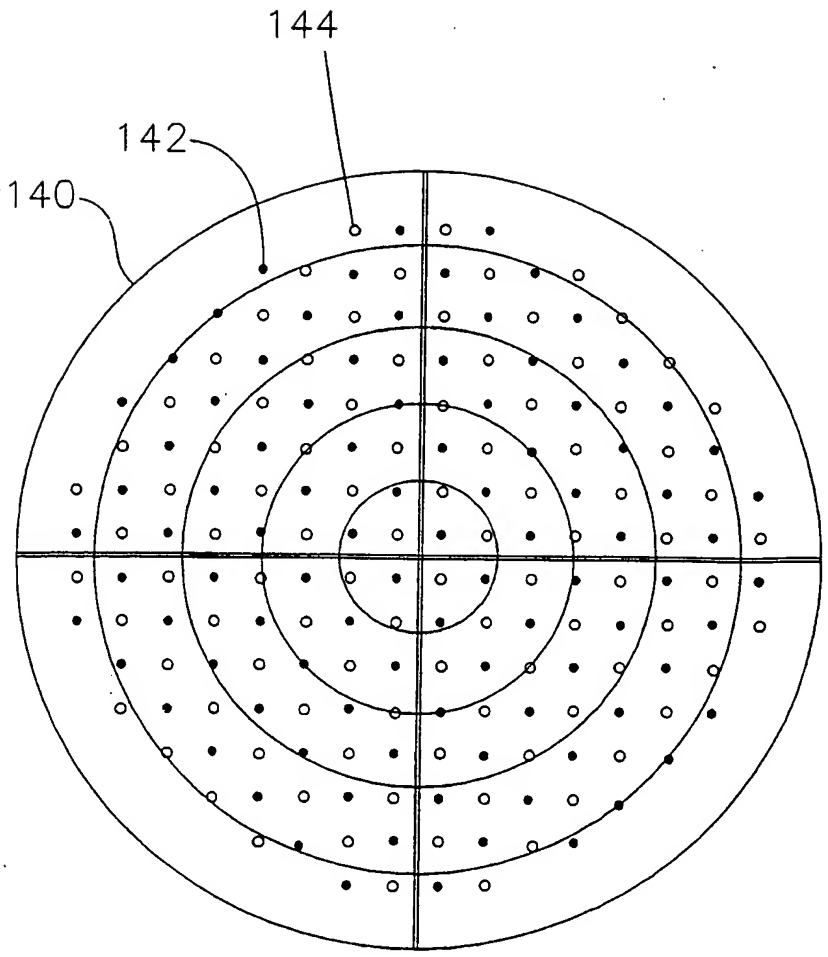


FIG. 7A

FIG. 7B

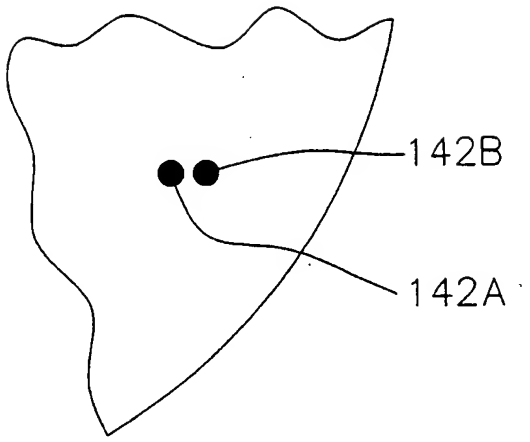


FIG. 7C

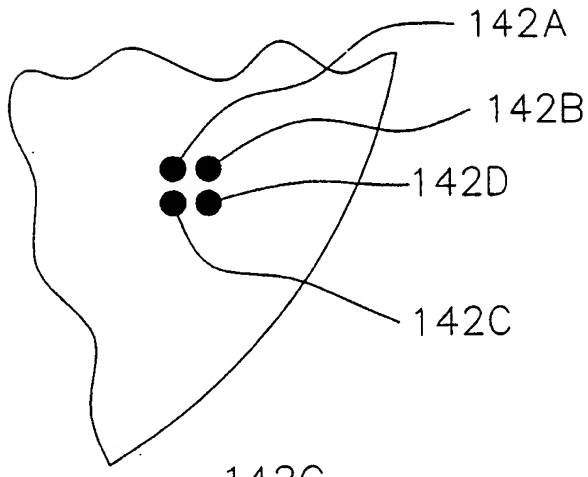
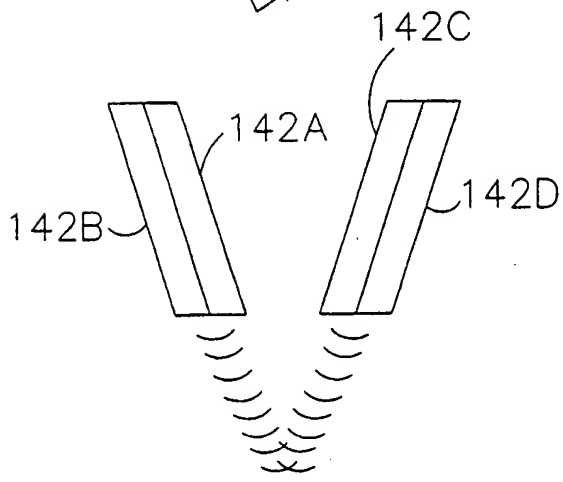


FIG. 7D



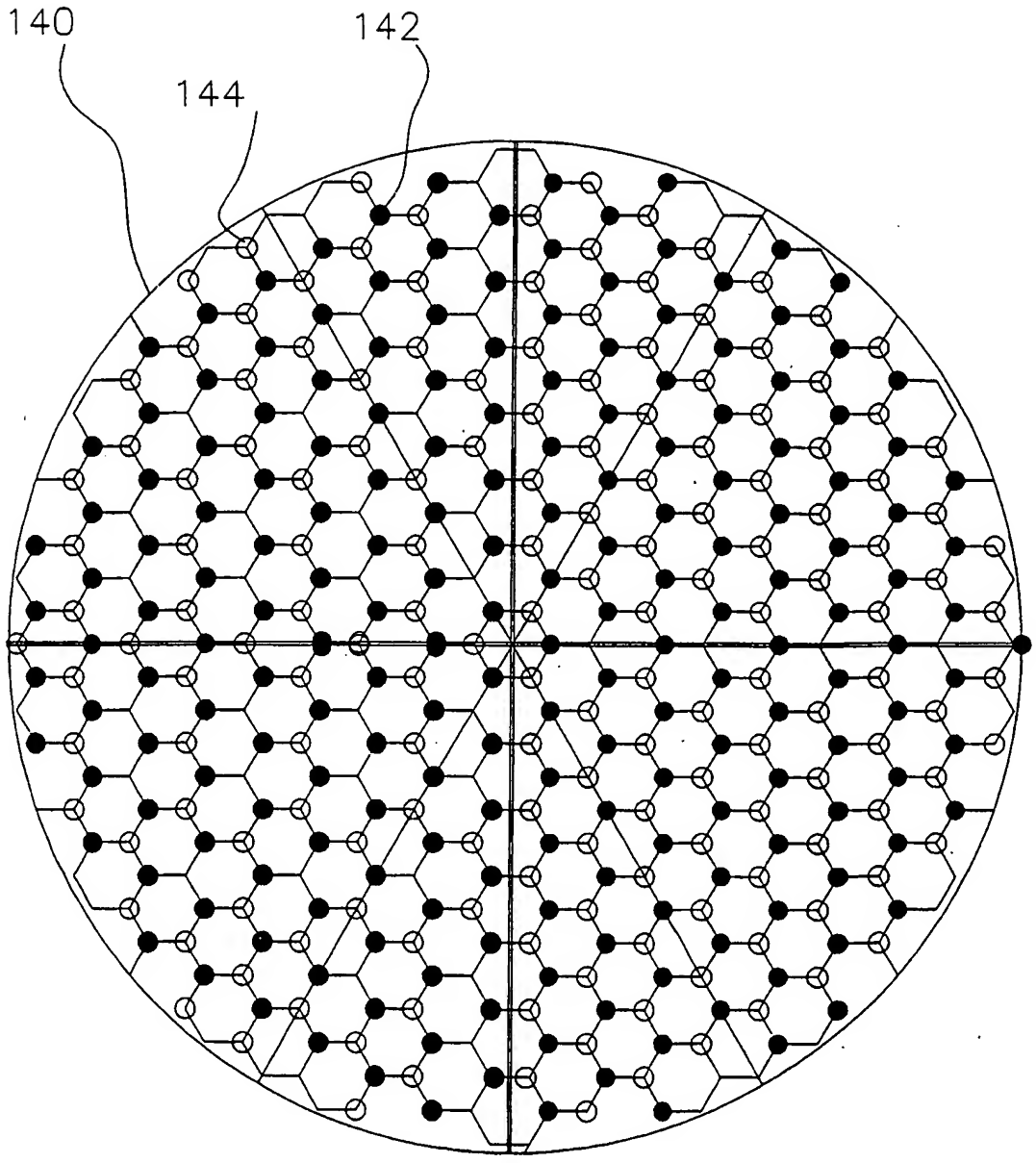


FIG. 8

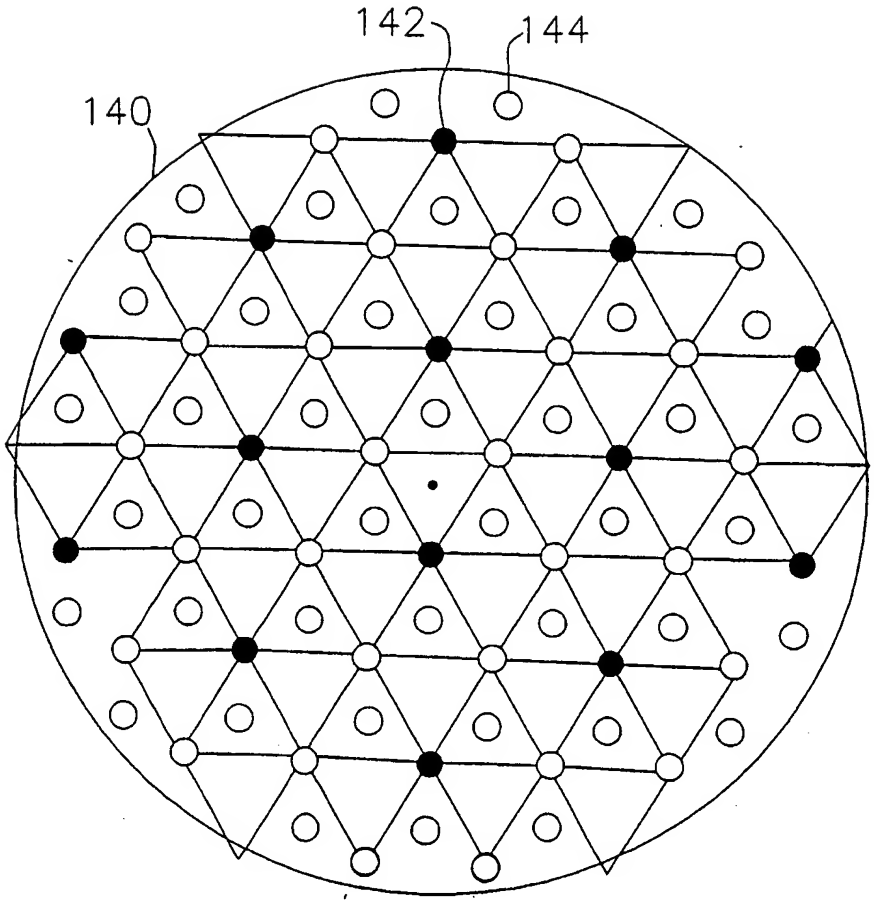


FIG. 9

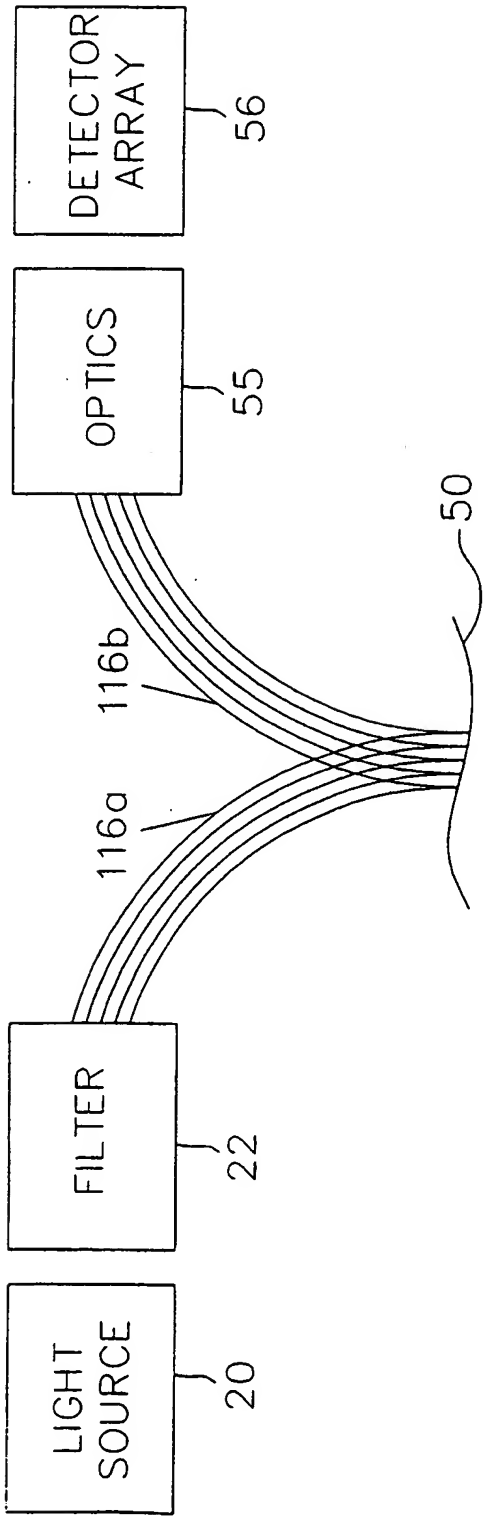

DETECTOR ARRAY
56
OPTICS
55
50
116b
116a
FILTER
22
LIGHT SOURCE
20

FIG. 10

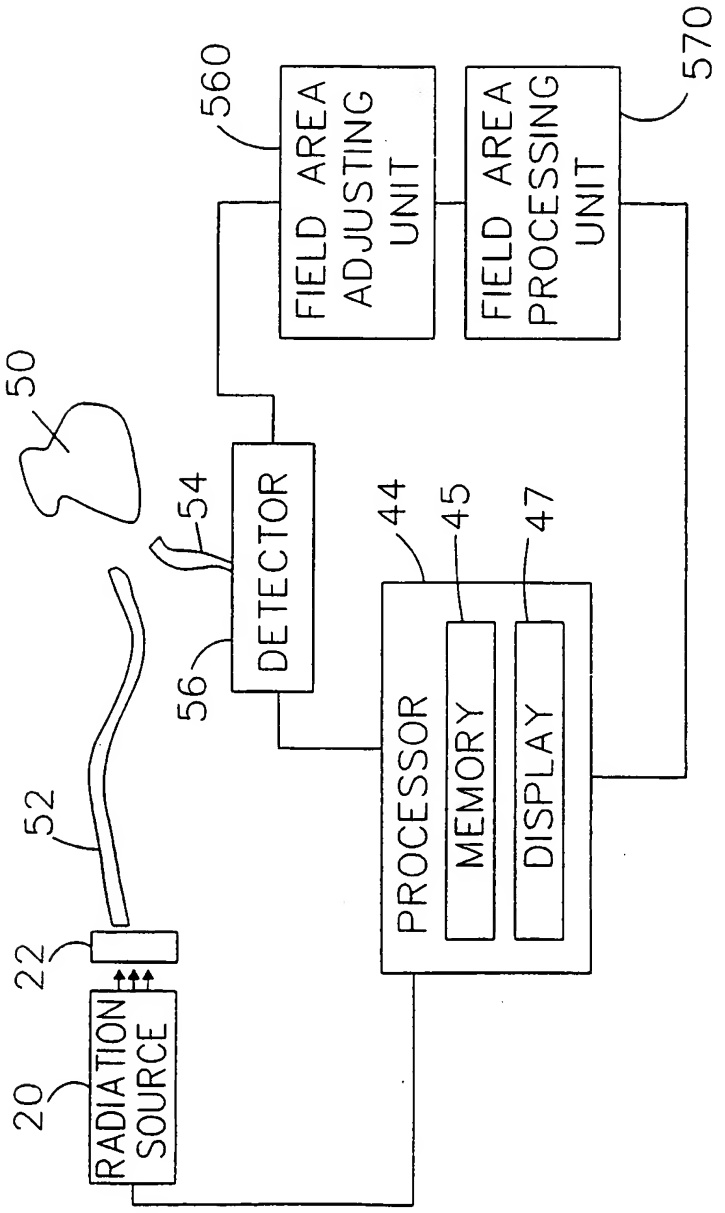

560
FIELD AREA ADJUSTING UNIT
570
FIELD AREA PROCESSING UNIT
50
54
DETECTOR
44
45
47
56
PROCESSOR
MEMORY
DISPLAY
52
22
20
RADIATION SOURCE

FIG. 11A

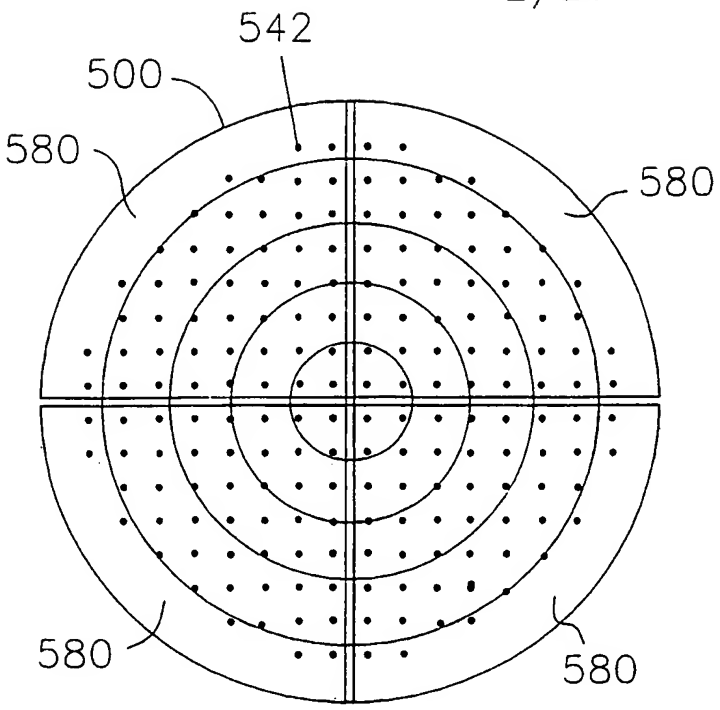


FIG. 11B

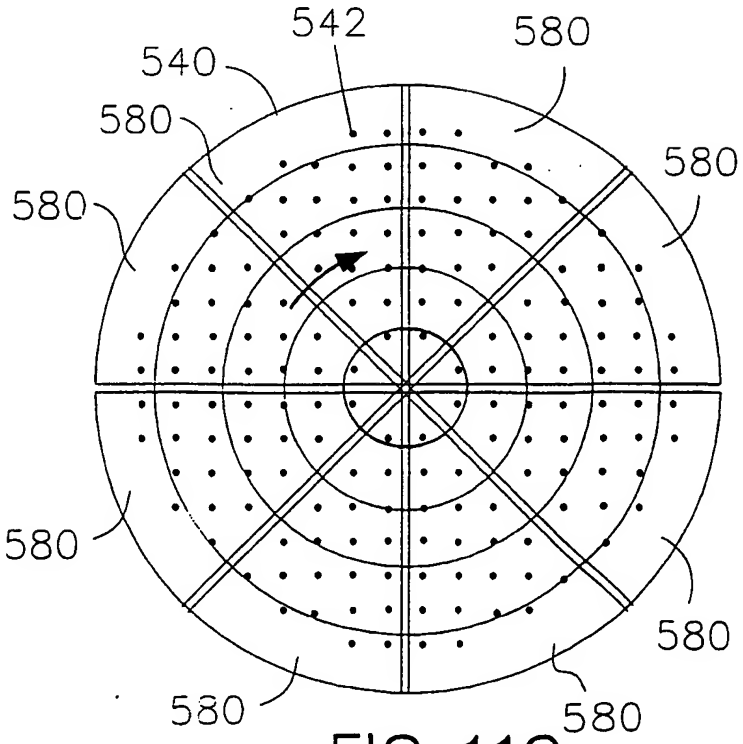


FIG. 11C

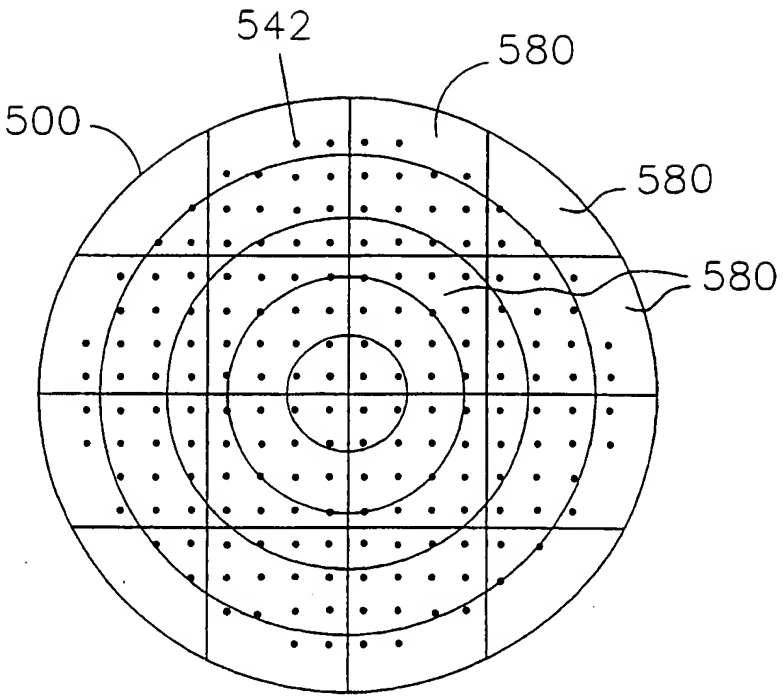


FIG. 11D

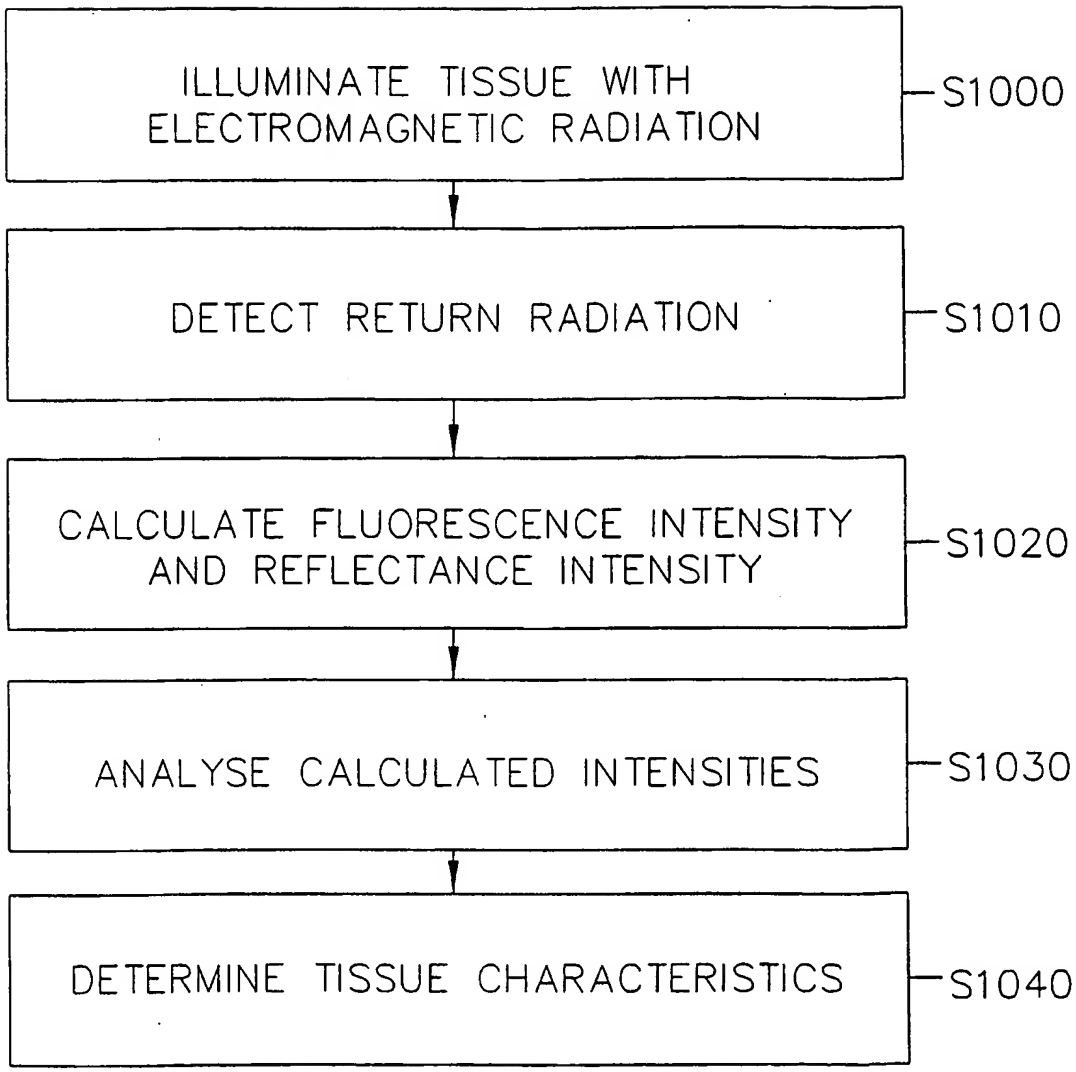

ILLUMINATE TISSUE WITH ELECTROMAGNETIC RADIATION
S1000
DETECT RETURN RADIATION
S1010
CALCULATE FLUORESCENCE INTENSITY AND REFLECTANCE INTENSITY
S1020
ANALYSE CALCULATED INTENSITIES
S1030
DETERMINE TISSUE CHARACTERISTICS
S1040

FIG. 12

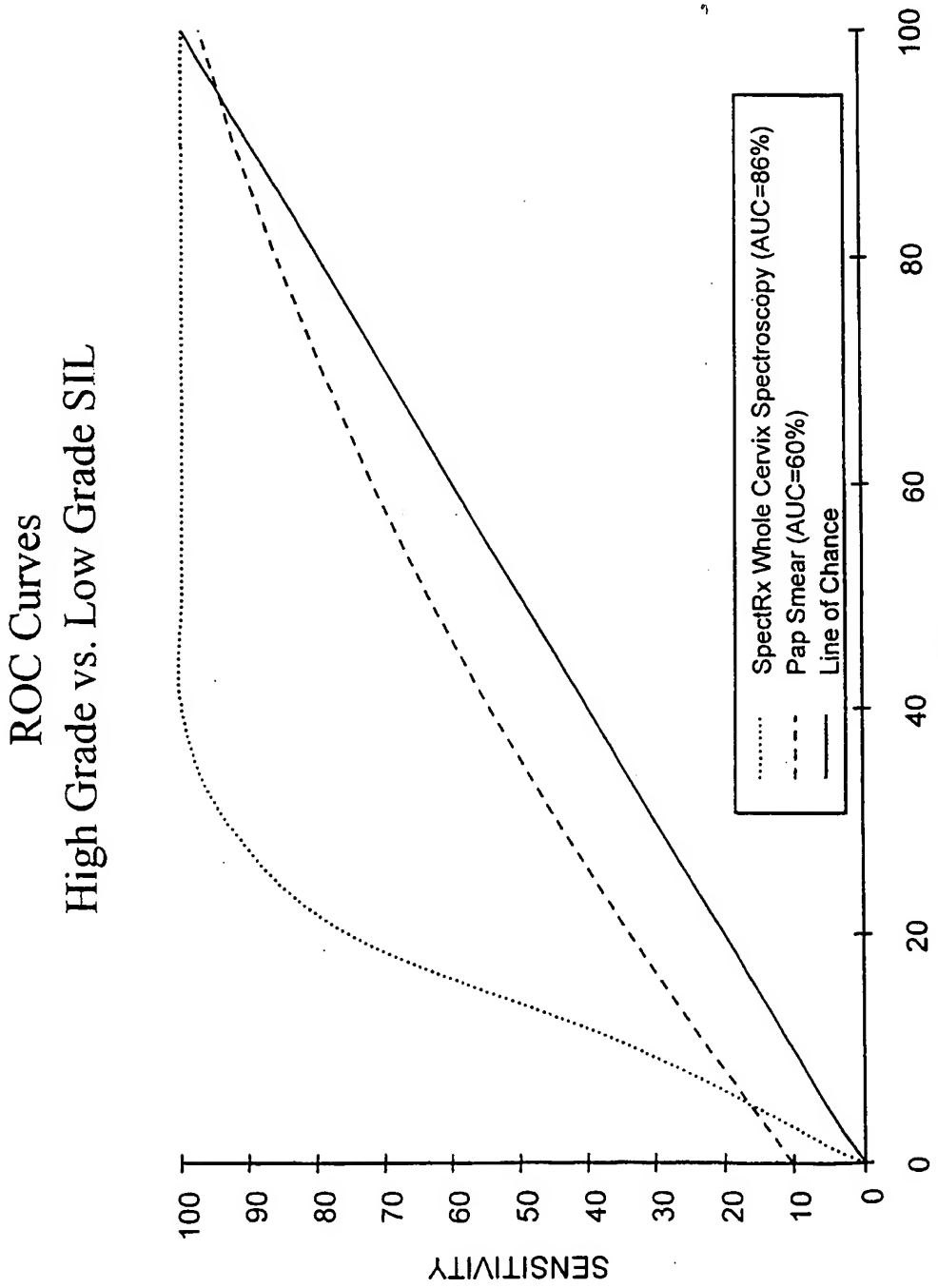

ROC Curves
High Grade vs. Low Grade SIL
SENSITIVITY
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
1-SPECIFICITY
SpectRx Whole Cervix Spectroscopy (AUC=86%)
Pap Smear (AUC=60%)
Line of Chance

FIG. 13

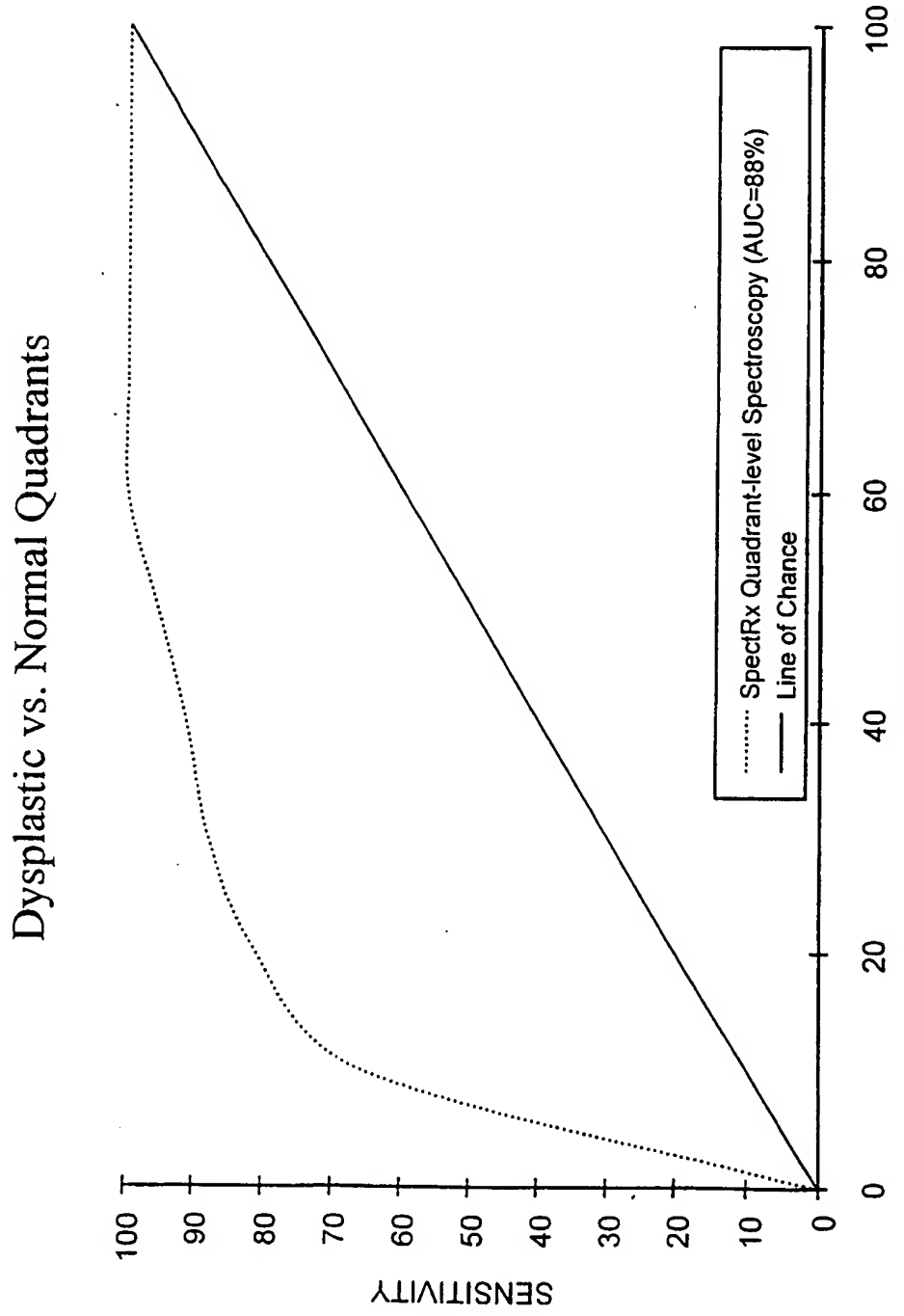
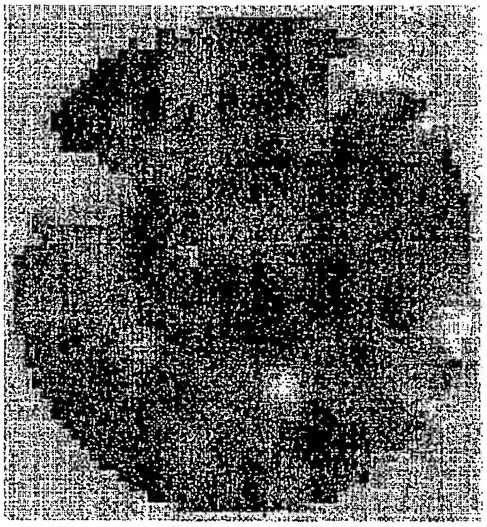

ROC Curve
Dysplastic vs. Normal Quadrants
SENSITIVITY
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
1-SPECIFICITY
SpectRx Quadrant-level Spectroscopy (AUC=88%)
Line of Chance

FIG. 14

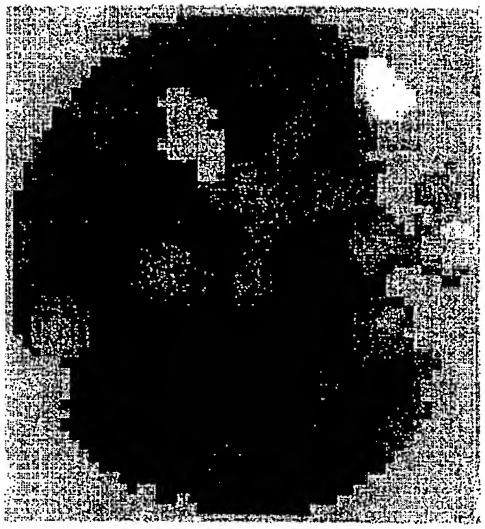
False Color Fiber Optic Maps
LG SIL



Patient 102-ST-CP-076-LW
Pap Test: LG SIL
Colposcopy: No Lesions
Pathology: LG SIL

FIG. 15A

False Color Fiber Optic Maps
HG SIL



Patient 101-TR-CP-007-SG
Pap Test: Reactive changes
Colposcopy: HG SIL
Pathology: HG SIL

FIG. 15B

MHI Camera System
HG SIL

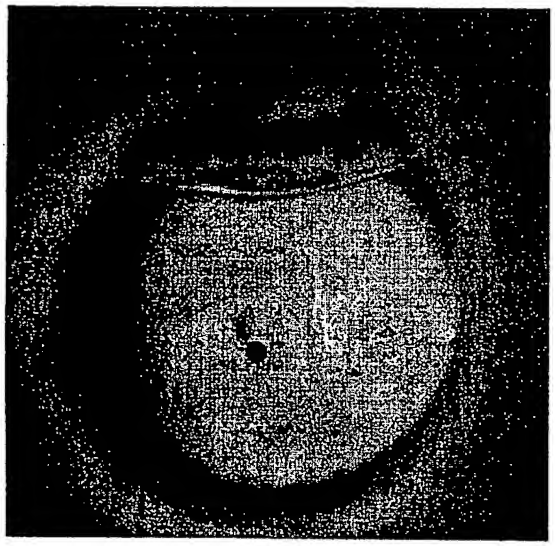


FIG. 16A

MHI Camera System
No Dysplasia

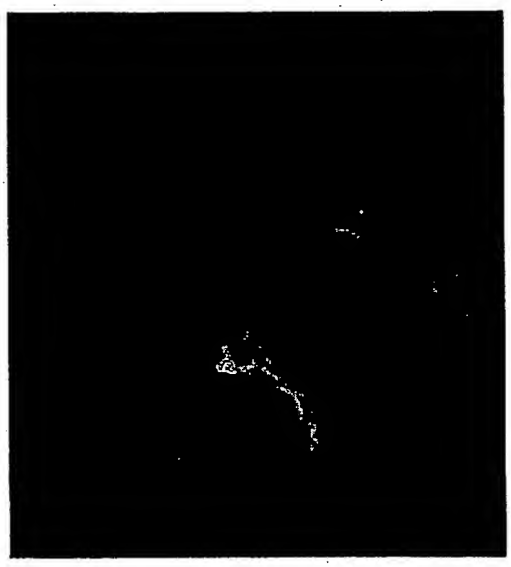


FIG. 16B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/28879

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61B 6/00
US CL :600/476
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 600/476, 477, 478, 310, 342; 250/341.3, 461.2; 356/369

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,582,168 A (SAMUELS et al.) 10 December 1996, see abstract, col. 1 line 20 - col. 2 line 6, col. 2 lines 18-21, col. 3 lines 13-49 and claims 1-19.	1-3, 5, 6, 19-24, 26, 36, 37 --- 10, 27, 33
Y	US 5,090,415 A (YAMASHITA et al.) 25 February 1992, see abstract, figure 1, col. 1 line 60 - col. 2 line 20.	10, 27, 33
A	US 5,596,992 A (HAALAND et al.) 28 January 1997, see abstract, and col. 1 line 66 - col. 2 line 21.	1-46
A	US 5,762,609 A (BENARON et al.) 09 June 1998, see abstract, and col. 5 line 20 - col. 6 line 8.	1-46

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JANUARY 2001	Date of mailing of the international search report 21 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHAWNA J. SHAW Telephone No. (703) 308-0858

Form PCT/ISA/210 (second sheet) (July 1998)★